Isoflurane

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 'Isofluraan'
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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 Isofluraan. Dit advies is opgesteld door de Commissie Reprotoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. Ik heb deze publicatie heden ter kennisname aan de Minister van Volksgezondheid, Welzijn en Sport en aan de Minister van de Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

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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. 2002/13OSH, The Hague, 6 september 2002

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

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

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

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Samenvatting

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

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte gegevens beschikbaar zijn. Zij adviseert daarom om isofluraan niet te classificeren.
- Voor effecten op de ontwikkeling is de commissie van mening dat er onvoldoende geschikte humane gegevens zijn en dat voldoende diergegevens laten zien dat isofluraan de ontwikkeling van het nageslacht niet schaadt. Zij adviseert daarom isofluraan niet te classificeren.
- Voor effecten tijdens lactatie, adviseert de commissie om isofluraan niet te kenmerken wegens onvoldoende geschikte 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 isoflurane.

The committee's recommendations are

- For effects on fertility, the committee recommends not to classify isoflurane due to a lack of appropriate human and animal data.
- For developmental toxicity, the committee is of the opinion that a lack of appropriate human data precludes the assessment of isoflurane and that sufficient animal data show that no classification is indicated
- For effects during lactation, the committee is of the opinion that a lack of appropriate data precludes the labeling of isoflurane.

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 labeled as 'may cause harm to breastfed babies' (R64).

1.2 Committee and procedure

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

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

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In 2002, the President of the Health Council released a draft of the report for public review. The individuals and organizations 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 labeling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breastmilk in amounts sufficient to cause concern for the health of a breastfed child, should be labeled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of the dosage), the labeling for effects during lactation is based on a risk characterization and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labeled 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 2000. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted as well as several websites regarding (publications on) toxicology and health. References are divided in literature cited and literature consulted but not cited.

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

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

1.6 Presentation of conclusions

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

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

1.7 Final remark

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

* for definitions see Tox95

Chapter 2

Isoflurane

2.1 Introduction

Name	:	Isoflurane
Chemical name		1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro ethane
Other name	:	forane
CAS-no	:	26675-46-7
Use	:	anaesthetic gas (since 1979)
Mol weight	:	184.50
Chem formula	:	$C_3H_2ClF_5O$
Conversion factor	:	1 ppm = 7.55 mg/m^3 (101 kPa, 25ºC) 1 mg/m^3 = 0.13 ppm 1% = 10000 ppm = 75500 mg/m^3

2.2 Human studies

Human studies are described in more detail in Tables 1 and 2 (annex D).

Fertility

Peelen *et al*. (Pee99) reported that time to pregnancy was not affected in operation chamber assistants (OR 0.9, 95% CI 0.6-1.4). In this study the concentrations of several of anaesthetic gasses were measured; the maximal isoflurane concentration measured was 120 mg/m^3. However, in this study, the composition of the anaesthetic gas mixtures, and the level and duration of exposure were not reported. Therefore, the committee is of the opinion that this study is no reason for classification.

Development

Johnson *et al*. (Joh87) did not find an increase in spontaneous abortion in a case control study among female veterinarians, veterinarian assistants and wives of male veterinarians exposed to a mixture of anaesthetics. No information was available on the concentration of the different anaesthetics.

In contrast, Guirguis *et al*. (Gui90) found an increase in spontaneous abortions and congenital anomalies among female hospital staff exposed to mixtures of anaesthetics (concentration unknown) (abortions: OR 1.98, 95% CI 1.53-2.56; anomalies: OR 2.24, 95% CI 1.69-2.97) and wives of males hospital staff exposed to a mixture of waste anaesthetics (abortions: OR 2.3, 95% CI 1.68-3.13; anomalies: OR 1.46, 95% CI 1.04-2.05). Odds ratios were adjusted for several confounders.

Peelen *et al*. (Pee99) studied the effects of exposure to anaesthetic gasses on the time to pregnancy, spontaneous abortions, preterm birth, low birth weight and congenital anomalies in operation personal. An increased risk for spontaneous abortion (OR 1.3, 95% CI 0.8-2.1), preterm birth (OR 1.9, 95% CI 1.2-3.0) and congenital abnormalities (OR 1.6, 95% CI 0.9-2.9) was observed. After correction for alcohol use, work circumstances and other occupational exposure, the OR for preterm birth was 1.4 (0.7-2.8) and the OR for congenital abnormalities 1.8 (1.0-4.1). Women present at the beginning of operations or present at tonsil operations have higher risks for spontaneous abortions (OR 1.6 (0.9-2.5)/ OR 1.9 (1.1-3.6) not corrected for confounders) and preterm birth (OR 2.0 (1.2-3.4)/ OR 1.6 (0.8-2.0) not corrected for confounders). In this study, the concentration of anaesthetic gasses was measured; the maximal isoflurane concentration measured was 120 mg/m^3.

However, in the abovementioned studies, hospital staff was exposed to a mixture of anaesthetic gasses. For that reason it was not clear if isoflurane caused the slight increases in reproductive effects. Therefore, the committee is of the opinion that none of the available studies could be used for classification purposes.

Lactation

Fisher *et al*. (Fis97) studied the human blood/air and milk/air partition coefficient (PC) in human blood and human milk samples (n=10). The objective of this study was to evaluate the potential chemical exposure of a nursing infant by ingestion of contaminated milk from a mother who was occupationally exposed to vapours; To estimate the infants' exposure, a generic human pharmacokinetic (PB-PK) lactation model was developed. The model was based on a 8-hour exposure period of the mother to a constant (isoflurane) vapour concentration of 50 ppm (384 mg/m^3). The experimentally determined blood/air and milk/air PC values were used in the PB-PK lactation model. The predicted amount of isoflurane ingested by a nursing infant over a 24-hour period was 0.336 mg in 0.92 l (0.37 mg/l). However, this model has not been validated yet and the relevance of this exposure level to the development of the human infant is unknown.

2.3 Animal studies

Fertility and developmental toxicity studies with isoflurane in experimental animals are summarized in Tables 3 and 4, respectively.

Fertility studies

Male (C57B1/C3H)F1 mice were exposed by inhalation to air, 0.1 and 1% [7550 and 75500 mg/m^3] isoflurane for 4 h/day for 5 days (Lan81). After 28 days epididymal spermatozoa were evaluated for morphological changes. The percentage abnormal spermatozoa in both isoflurane-exposed groups was comparable to the control group (1.70 $\pm$ 0.18 (1%), 1.20 $\pm$ 0.24 (0.1%) versus 1.44 $\pm$ 0.19 in the control). Mortality was not observed. General toxicity was not described in this study.

No effects on pregnancy rate, number of implantation, resorptions or live foetuses were observed when untreated female Swiss/Webster mice were mated with males exposed to air, 0.1 and 0.4% [7550 and 30200 mg/m^3] isoflurane 4h/day for 6 weeks prior to mating or when both males and females were treated 2 weeks prior to mating and during mating and gestation (Maz85a). No toxicity was observed; the exposure to the highest concentration resulted in light anaesthesia.

Developmental toxicity

After exposure of both male and female Swiss/Webster mice to 0.1 and 0.4% [7550 and 30200 mg/m^3] isoflurane 4h/day 2 weeks prior to mating and during mating and gestation no differences were found in the number of live foetuses per litter, the viability and lactation index and pup weight (Maz85a). In this study no toxicity was found; mice exposed to the highest concentration were slightly sedated.

Exposure of female Swiss/Webster mice during GD 6-15 to 0.006, 0.06 and 0.6% [450, 4500 and 45000 mg/m^3] isoflurane 4h/day did not result in any effects on litter size and sex ratio (Maz85b). However, at the highest concentration an increase in late resportions, a decrease in litter weight and skeletal ossification as well as an increase in cleft palate and renal pelvic cavitation was observed. Dams exposed to the highest concentration isoflurane showed ataxia followed by light general anaesthesia and a lower weight gain, partly due to a decreased litter weight.

Mazze *et al*. (Maz86) found decreased foetal weight after exposure of Sprague Dawley rats from GD 8-10 or 14-16 to 1.05% [78750 mg/m^3] isoflurane for 6 h/day by inhalation. Dams suffered from light general anaesthesia and decreased weight gain.

Foetal weight was not affected after exposure of female rats exposed from GD 11-13 to 1.05% isoflurane. The dams suffered from light general anaesthesia.

Exposure of Sprague Dawley rats on GD 8 to 0.35% [26425 mg/m^3] isoflurane for 24h did not result in any effect on the number of implantations, live foetuses, resorptions, mean foetal body weight or sex ratio (Fuj87). Moreover, in external, visceral and skeletal examinations no differences were found. Maternal mortality was not observed, but the rats were slightly sedated during exposure and had lower body weights on GD 12, 14 and 16.

Lactation

No publications were available.

2.4 Conclusion

Only one human study on effects on fertility was available which showed that the time to pregnancy was not affected in operation chamber assistants exposed to (mixtures of) anaesthetics (Pee99). However, the anaesthetic gases used and the concentrations were not specified.

In an animal study, male mice were inhalatory exposed to high concentrations of isoflurane for 5 days and sperm morphology was studied 28 days after the last day of exposure (Lan81). No changes in sperm morphology were observed. However, as the spermatogenic cycle of mice takes 56 days, the exposure and latency periods of the experiment were too short to be able to detect effects on all stages of spermatogenesis. Inhalatory exposure of male Swiss/Webster mice mated with untreated females or treatment of both males and females to a maximum of 0.4% [30200 mg/m^3] isoflurane did not result in reproductive effects (Maz85a).

In conclusion, that committee is of the opinion that a lack of appropriate human and animal data precludes the assessment of isoflurane for fertility.

Two out of three epidemiological studies reported developmental effects after exposure of (veterinarian) hospital staff to anaesthetic waste (Gui90, Pee99). However, the hospital staff was exposed to mixture of anaesthetics. Therefore, the committee is of the opinion that a lack of appropriate human data precludes the assessment of isoflurane for development

In animals, effects on foetal weight were found in progeny of Sprague Dawley rats exposed inhalatory to 1.05% [78750 mg/m^3] isoflurane (Maz86). Progeny of Swiss/Webster mice exposed to 0.6% [45000 mg/m^3] isoflurane showed an increase in late resorptions, a decrease in litter weight and skeletal ossification, as well as an

increase in cleft palate and renal pelvic cavitation (Maz85b). However, in both studies maternal toxicity (weight loss) was observed. Two other studies in Swiss/Webster mice and Sprague Dawley rats failed to show any effect of inhalatory exposure to a maximum of 0.4% [30200 mg/m^3] isoflurane on development (Maz85a, Fuj87).

In view of these data, the committee is of the opinion that sufficient animal data show that no classification for effects on development is indicated.

From the study of Fisher *et al* (a pharmacokinetic lactation model), an amount of 0.37 mg isoflurane per liter breast milk was predicted (Fis97). The committee is of the opinion that this (predicted) isoflurane concentration in human breast milk can only be used as an indication for the possible amount of the compound in breast milk, because the model is not yet sufficiently validated. The committee concluded that a predicted exposure level per se is not a sufficient basis for labeling isoflurane. No experimental data are available about the concentration of isoflurane in human breast milk and about the possible effects during lactation. In conclusion, the committee proposes not to label isoflurane for effects during lactation because of a lack of appropriate data.

Proposed classification for fertility

Lack of appropriate human and animal data precludes assessment of isoflurane for fertility.

Proposed classification for developmental toxicity

Lack of appropriate human data precludes assessment of isoflurane for development and sufficient animal data show that no classification is indicated.

Proposed labelling for effect during lactation

Lack of appropriate data precludes assessment of isoflurane for labeling for effects during lactation

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Annexes

Annex **A**

The committee

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Annex **B**

Comments on the public draft

A draft of the present report was released in 2002. The following persons and organisations have commented on the draft review:

- A Aalto
  Ministry of Social Affairs and Health, Finland
- RD Zumwalde
  National Institute for Occupational Safety and Health, USA

Annex **C**

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

4.2.3 Substances toxic to reproduction

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

Category 1:

Substances known to impair fertility in humans

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

Substances known to cause developmental toxicity in humans

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

Category 2:

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

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

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

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

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

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

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

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

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

Generally on the basis of:

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

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

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

T; R60: May impair fertility

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

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

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

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

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

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

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

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

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

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

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

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

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

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

Effects on fertility

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

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

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

Developmental toxicity

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

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

a) toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk, and/or
b) on the basis of results of one or two generation studies in animals which in- dicate the presence of adverse effects on the offspring due to transfer in the milk, and/or
c) on the basis of evidence in humans indicating a risk to babies during the lactational period. Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and developmental toxicity studies

Table 1 Fertility studies in man.

authors	exposure	study type/data collection	study/comparation population	investigated effects and results	remarks
Pee99	Mixture: waste anaesthetics Concentration isoflurane measured maximal 120 mg/m^3; Employment in anaesthesia	Retrospective survey The Netherlands 1990-1997/postal questionnaire	427 pregnant females (age 22-37 years) employed in anaesthesia/ 1,010 pregnant females (age 22-37 years) nurses employed in department of orthopaedics, gynaecology or surgery	No effect on time to pregnancy	Mixed exposure

Table 2 Developmental toxicity studies in man.

authors	exposure	study type/data collection	study/comparation population	investigated effects and results	remarks
Joh87	Mixture: waste anaesthetics	Case-control USA/postal questionnaire (additional questionnaire sent to senior female veterinary assistants)	278 spontaneous abortions and stillbirths and 98 live birth with congenital abnormalities that occurred to female veterinarians and veterinarian assistants and wives of male veterinarians 642 normal pregnancies chosen on a stratified random basis	No statistically significant increase in spontaneous abortions	Mixed exposure Results adjusted for x-ray exposure
Gui90	Mixture: waste anaesthetics	Retrospective study Ontario 1981-1985/ questionnaire	Exposed (n=6336) and non-exposed (n=2202) hospital staff and their or their wives pregnancies/children	Significant increase in spontaneous abortion and congenital abnormality among exposed females (OR 1.98, 95% CI 1.53-2.56; OR 2.24, 95% CI 1.69-2.97) and spouses of exposed males (OR 2.3, 95% CI 1.68-3.13; OR 1.46, 95% CI 1.04-2.05)	Mixed exposure OR's were standardised for age, smoking, alcohol consumption, previous abortion and occupation
Pee99	Mixture: waste anaesthetics Concentration isoflurane measured maximal 120 mg/m^3. Employment in anaesthesia	Retrospective survey The Netherlands 1990-1997/postal questionnaire	427 pregnant females (age 22-37 years) employed in anaesthesia/1,010 pregnant females (age 22-37 years) nurses employed in department of orthopaedics, gynaecology or surgery	Increased risk for abortion preterm birth and congenital abnormalities	Mixed exposure *Controlled for age, education, menstrual cycle, life style and circumstances during work.

Table 3 Fertility studies in animals with isoflurane.

authors	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs and reproduction	remarks
Lan81	male (C57B1/C3H)F 1 mice (n=5)	4h/day during 5 consecutive week days sacrifice 28 days after start of exposure; sperm morphology of both cauda epididymides	0, 0.1 and 1% [7550 and 75500 mg/m^{3}] isoflurane inhalation	no paternal mortality and paternal toxicity described	no change in percentage of abnormal	
Maz85a	male Swiss/Webster mice (n=15-24)	4h/day daily for 7 weeks after 6 weeks treated males were mated with untreated females uterine content of females was examined on GD 18	0, 0.1 and 0.4% [7550 and 30200 mg/m^{3}] isoflurane inhalation	all mice survived and weight gain was identical for all groups exposure to highest concentration resulted in light anaesthesia	no effects on male fertility all males became sire no effects on pregnancy rate, number of implantations, resorptions or dead in utero	
Maz85a	male and female Swiss/Webster mice (n=32-41)	4h/day daily for 2 weeks after 2 weeks males were mated with females of the same group exposure was continued during mating and pregnancy uterine of two third of the females was examined on GD 18; one third of the females littered and raised their pups	0, 0.1 and 0.4% [7550 and 30200 mg/m^{3}] isoflurane inhalation	all mice survived and weight gain was identical for all groups exposure to highest concentration resulted in light anaesthesia	no effects on fertility no effects were found on pregnancy rate, number of implantations, live foetuses and resorptions	

Table 4 Developmental toxicity studies in animals with isoflurane.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Maz85a	male and female Swiss/ Webster mice (n=32-41)	4h/day daily for 2 weeks after 2 weeks males were mated with females of the same group exposure was continued during mating and pregnancy uterine of two third of the females was examined on GD 18 one third of the females littered and raised their pups	0, 0.1 and 0.4% [7550 and 30200 mg/m^3] isoflurane inhalation	all mice survived and weight gain was identical for all groups exposure to highest concentration resulted in light anaesthesia	no differences in live foetuses/litter, viability and lactation index and pup weights from PN1 to PN28	
Maz85b	female Swiss/ Webster mice (n=23-31)	4h/day on GD 6-15 Caesarean section on GD 18	0, 0.006, 0.06 and 0.6% [453, 4530 and 45300 mg/m^3] isoflurane inhalation	highest concentration: lower weight gain*, ataxia followed by light general anaesthesia	no effects on litter size or sex ratio at highest concentration: decreased litter weight, increase in late resorptions, cleft palate and renal pelvic cavitation, decreased skeletal ossification	*in part related to decreased litter weight
Maz86	pregant Sprague Dawley rats (n=39-50 control, 21-25 isoflurane)	exposure 6h/day on I. GD 14-16 II. GD 11-13 III. GD 8-10 screening for skeletal and soft tissue anomalies after Caesarean section	0 and 1.05% [79275 mg/m^3] isoflurane inhalation	I, II and III: light anaesthesiaI and III: decreased weight gain	I and III: decreased foetal weight	
Fuj87	pregnant Sprague Dawley rats (n=40 control, 30 isoflurane)	exposure on GD 8 for 24 h Caesarean section on GD 20	0 and 0.35% [26425 mg/m^3] isoflurane inhalation	no maternal mortality during exposure rats were mildly sedated at GD12, 14 and 16 rats weighed less than control rats	no influence on number of implantations, live and dead foetuses, resorptions, foetal body weight and sex ratio no differences in external, visceral and skeletal abnormalities	

Annex **E**

Abbreviations

Abbreviations used:

bw	=	body weight
CI	=	confidence interval
CNS	=	central nervous system
d	=	day
F	=	female(s)
GD	=	gestation day
i.p.	=	intraperitoneal
IRPC	=	increased renal pelvic cavitation
i.v.	=	intravenous
M	=	male(s)
n	=	number
NOAEL	=	no adverse effect level
OECD	=	Organisation for Economic Cooperation and Development
OR	=	Odds ratio
OT	=	Operating theatre
PN	=	postnatal
RR	=	relative risk