Halothane

Evaluation of the effects on reproduction, recommendation for classification

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

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Mijnheer de Staatssecretaris,

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

In 1996 heb ik hiervoor de Commissie Reproductietoxische stoffen ingesteld.

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

Hoogachtend,
w.g.
prof. dr JJ Sixma

Halothane

Evaluation of the effects on reproduction, recommendation for classification

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

to

the Minister and State Secretary of Social Affairs and Employment

No. 2000/02OSH, The Hague, 1 May 2000

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie van reproductie toxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie halothaan onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit meent de commissie dat er onvoldoende gegevens beschikbaar zijn. Zij adviseert daarom halothaan niet te classificeren.
- Voor ontwikkelingsstoornissen, adviseert de commissie om halothaan in categorie 3 (*Stoffen die in verband met hun mogelijke voor de ontwikkeling schadelijke effecten reden geven tot bezorgdheid voor de mens*) te classificeren en met R63 (*mogelijk gevaar voor beschadiging van het ongeboren kind*) te kenmerken.
- Voor effecten tijdens de lactatie, adviseert de commissie om halothaan *niet* te kenmerken wegens gebrek aan bewijs.

Executive summary

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

The committee's recommendations are:

- For effects on fertility, the committee recommends no classification of halothane, due to a lack of appropriate data.
- For developmental toxicity, the committee recommends to classify halothane in category 3 (*Substances which cause concern for humans owing to possible developmental toxic effects*) and to label halothane with R63 (*possible risk of harm to the unborn child*).
- For effects during lactation, the committee is of the opinion that due to a lack of appropriate data halothane should *not* be labelled with R64.

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 according to the guidelines of the European Union (Directive 93/21/EEC) by the Health Council's Committee for Compounds Toxic to Reproduction. The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1 and 2) of the European Union.

1.2 Committee and procedure

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

Classification 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	
Labeling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In June 1999, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

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

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

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

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk 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 compound as potentially toxic to the breastfed child when exposure to this compound via the milk results in an intake exceeding an exposure limit for the general population, eg the acceptable daily intake (ADI).

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up 1995. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection

* Organisation for Economic Cooperation and Development

of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1997. The results of this search were no reason for the committee to adjust the recommendations.

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

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

1.6 Presentation of conclusions

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

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

1.7 Final remark

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

* For definition see Tox95

Chapter 2

Halothane

2.1 Introduction

Halothane is a non-flammable, highly volatile liquid with a sweetish odour.

Name:	:	2-bromo-2-chloro-1,1,1,-trifluoroethane
CAS reg No.	:	151-67-7
Use	:	anaesthetic gas
Mol weight	:	197.38
Chem formula	:	C_2BrClF_3H
Conversion factors (101 kPa; 20ºC)	:	1 ppm = 8.21 mg/m^3 1 mg/m^3 = 0.122 ppm

2.2 Human studies

Fertility

Andersen *et al* did not find differences in sperm quality in subjects before and after exposure to halothane in combination with nitrous oxide (And92).

In a recent study Peelen *et al*. (1999) reported that the time to pregnancy was not affected in operation chamber assistants (Pee99). In this study the concentrations of sever-

al of anaesthetic gases were measured; the maximal halothane concentration measured was 135 mg/m^3 (~16 ppm).

Developmental toxicity

Several epidemiological studies were performed in which female anaesthetists, operating nurses and wives of male anaesthetists were inquired about the course and outcome of their pregnancies, with specific attention for miscarriages and congenital anomalies (Ask70, Coh71, Ame74, Cor74, Kni72, Kni75, Pha77, Sch77, Ros78, Eri79). In all studies, except Ericson *et al* (1979), some effects on these parameters were suggested (Eri79). However, the studies were criticised by several authors (Wal75, Fer78, Ves78, Dud81) for the following reasons: studies were retrospective and often loaded questionnaires were used. Age differences occurred between the exposed and control group and no consideration was given to social factors, medication, illnesses and possible stress. Furthermore, the composition of the anaesthetic gas mixtures, in which halothane was one component, the duration of exposure and its timing in pregnancy, were often not reported.

For these reasons, the committee considers the quality of these studies insufficient for definite conclusions about the causality of the observed association between human exposure to halothane and adverse pregnancy outcome.

In a recent study, Peelen *et al*. (1999) studied the effects of exposure to anaesthetic gasses on time to pregnancy, spontaneous abortions, preterm birth, low birth weight and congenital anomalies in operation personnel (Pee99). An increased risk for spontaneous abortion (OR (odds ratio) =1.3), preterm birth (OR=1.9) and congenital abnormalities (OR=1.6) was observed. After correction for alcohol use, work circumstances and other environmental exposure on the working place, the OR for preterm birth was 1.4 and the OR for congenital abnormalities was 1.8. In this study, the concentration of anaesthetic gasses was measured; the maximal halothane concentration measured was 135 mg/m^3. However, operation personal was exposed to a mixture of anaesthetic gasses. For that reason, it was not clear if halothane caused the slight increases in reproductive effects.

Lactation

Coté *et al* (1976) detected halothane concentrations of 2 ppm in breast milk (mg/kg) of one lactating, practising anesthetist. This concentration was consistent with the operating theatre environment (Cot76). For analytical and methodological reasons, the concentrations measured in breast milk were regarded as an underestimated rather than an overestimated value. An earlier study indicated that respiratory excretion of halothane by operating theater personnel may continue for up to 72 hours after routine exposure (Cor73).

Fisher *et al*. 1997 studied the human blood/air and milk/air partition coefficient (PC) in blood and milk samples donated by lactating women (n=9) (Fis97). The objective of this study was to evaluate the potential chemical exposure of a nursing infant from ingestation of contaminated milk from a mother who was occupationally exposed to vapours. To estimate infants' exposure, a generic human pharmacokinetic (PB-PK) lactation model was developed. The model was based on a 8-hour exposure of the mother to a constant vapour concentration corresponding to the threshold limit value for halothane (50 ppm) in drinking water. The experimentally determined blood/air and milk/air PC values were used in the PB-PK lactation model. The predicted amount of halothane ingested by a nursing infant over a 24-hour period was 0.232 mg. However, this model has not been validated yet and the relevance of this exposure level to the development of the human infant is unknown.

2.3 Animal studies

Fertility and developmental toxicity studies with halothane in experimental animals are summarised in Tables 1, 2.1, 2.2 and 2.3.

Fertility

After inhalatory exposure of male rats, for 36 or 64 days prior to mating (mated to unexposed females), to 10 ppm halothane (~82 mg/m^3) for 8h/day during 5 days/week, no remarkable effects were observed on foetal parameters and reproduction (Hal81). Exposure of female rats, for 31 days prior to mating (mated to unexposed males) and during gestation, to 10 ppm for 8h/day during 5 days/week did not result in an effect on foetal parameters or female fertility (Hal81).

Developmental toxicity

Lesions in the kidney, liver and brain of 1 day old rat pups were observed in several studies (Cha75a, Cha75b, Cha76, Cha77 and Dud77) after inhalatory exposure to 10 ppm (~82 mg/m^3) halothane during 8h/day, 5d/week during gestation. Moreover, degenerative changes were still observable in the neurones of 100-day old F1-animals exposed in utero. No information about maternal toxicity was available.

Behavioural changes were observed in rats after pre- and postnatal exposure by inhalation to 10 ppm (~82 mg/m^3) halothane (Qui74, Qui75, Bow77, Dud77) and at 12.5 ppm (~103 mg/m^3) (Lev86) and 100 ppm (~820 mg/m^3) (Lev90).

Koëter *et al* studied the effects of pre-and postnatal exposure to 0.5% halothane (5000 ppm) in mice and observed retarded physical development (prenatal exposure) and

air-and surface righting (pre-and postnatal exposure) and affected locomotion (pre- and postnatal) (Koe80).

Mazze *et al* found developmental effects (decreased foetal weight and increased renal pelvic cavitation) in the presence of light maternal anaesthesia after exposure to 0.8% halothane (8000 ppm) 6 hours/day by inhalation during several intervals of gestation (Maz86).

In non-pregnant animals general toxicity was observed at 15 ppm (~123 mg/m^3) (Ste75: mice, liver degenerative lesions) and 20 ppm (164 mg/m^3) (Plu86: rats, increased liver weight). In the studies for developmental effects, information concerning general toxicity was not available. The effects in the developmental toxicity studies were observed at 10 ppm (~82 mg/m^3) (Cha75a, Cha75b, Cha76, Cha77, Dud77).

Lactation

No animal studies concerning the effects of halothane during lactation were available.

2.4 Conclusion

In man, the effects of halothane on fertility have been insufficiently studied. In an inhalation study in the rat a low concentration halothane (10 ppm, ~82 mg/m^3) did not result in an effect on male or female fertility. In view of these data, the lack of appropriate data preclude the assessment for fertility and therefore halothane should not be classified.

Epidemiological studies gave rise to concern about the effect of anaesthetic gas mixtures containing halothane on foetal development and miscarriages. However, it was not clear whether or not the effects described were caused by halothane. Confounding factors such as exposure to gas mixtures, differences in age between the control and the exposed groups and stress may have played a role. For that reason the quality of the human studies was considered insufficient for classification.

In several studies in rats and mice, behavioural effects and lesions of kidney, liver and brain were observed after pre- and postnatal exposure to halothane by inhalation. In non-pregnant animals general toxicity was observed at 15 ppm (123 mg/m^3) (Ste75: mice, liver degenerative lesions) and 20 ppm (164 mg/m^3) (Plu86: rats, increased liver weight). In the studies for developmental effects, information concerning general toxicity was not available. The effects in the developmental toxicity were observed at 10 ppm (~82 mg/m^3) (Cha75a, Cha75b, Cha76, Cha77, Dud77). Therefore, in view of the animal data with respect to the effects on development, the committee recommends to classify halothane in category 3 (’substances which cause concern for humans owing to pos-

sible developmental toxic effects'). Halothane should be labelled with R63 (Possible risk of harm to the unborn child).

Only two limited studies are available concerning the concentration of halothane on human breast milk (Cot76, Fis97). No data are available to assess the effects during lactation. For that reason the committee recommends not to label halothane for effects during lactation because of a lack of appropriate data.

Proposed classification for effects on fertility

The lack of appropriate data preclude the assessment of halothane for fertility.

Proposed classification for developmental toxicity

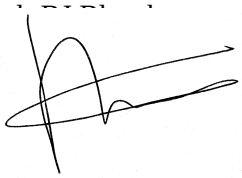
Category 3, R63.

Proposed labelling for effects during lactation

The lack of appropriate data preclude the labelling of halothane.

For the committee,
The Hague, 1 May 2000

dr ASAM van der Burght,
scientific secretary



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A The committee

B Comments on the public draft

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

D Fertility and Developmental toxicity studies

E Abbreviations

Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
  Toxicologist; Research Institute of Toxicology, Utrecht
- JN van den Anker
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- AM Bongers, *advisor*
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Secretarial assistance: E Vandenbussche-Parméus.
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 1999 for public review. No persons and organisations have commented on the public draft.

Annex **C**

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

4.2.3 Substances toxic to reproduction

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

Category 1:

Substances known to impair fertility in humans

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

Substances known to cause developmental toxicity in humans

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

Category 2:

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

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

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

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

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

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

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

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

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

Generally on the basis of:

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

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

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

T; R60: May impair fertility

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

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

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

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

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

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

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

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

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

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

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

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

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

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

Effects on fertility

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

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

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

Developmental toxicity

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

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

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

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

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

Annex **D**

Fertility and Developmental toxicity studies

Table 1 Fertility studies in animals with halothane.

authors	species/ route	experimental period/design	dose (ppm) and route	toxicity (general, parental, paternal or maternal)	effects on reproductive organs/effects on reproduction	remarks
Halsey *et al*. (1981)	Sprague Dawley rat (M, n=20)	exposure of males for 36 or 64 days prior to mating to unexposed females; sacrifice: females on GD21 and examination of foetuses sacrifice: males after 107 days exposure	0 and 10 ppm, 8h/d, 5d/w (inhalation)	no paternal toxicity at necropsy	No observable effects on foetal data in the halothane exposed groups.	Male fertility was not present- ed; as no statistical difference in the number of pregnant females was observed, it may be assumed that no effects on male fertility were present. Some females were allowed to litter and the offspring was mated. However, the number of animals used for this part of the studfy (n=3) was too small.
Halsey *et al*. (1981)	Sprague Dawley rat (F, n=20)	exposure of females for 31 days prior to mating, mated to unexposed males and exposed during gestation; sacrifice: females on GD21 and examination of	0 and 10 ppm, 8h/d, 5d/w (inhalation)	increased weight gain from GD1-21, no effect on female fertility	no observable effects on foetal data in the halothane exposed group	

Table 2.1 Developmental toxicity studies in animals with halothane.

authors	species	experimental period/design	dose (ppm) and route	maternal toxicity	developmental toxicity	remarks
Quimby *et al*. (1974 and 1975), Bowman and Smith (1977), Dudley (1977)	Sprague Dawley rats (F/litters, n=10-12)	exposure females and their offspring UU= undosed DU= exposure GD0-PN60 UD= PN60-PN135 (312 experiment 3) DD= GD0-PN135 (312 experiment 3); behavioural tests in male offspring of second generation Qui74 and Qui75; Bow77 exposed the grandparents and parents to the above DU, UD and DD conditions and tested the third generation males	0 and 10 ppm 8h/d, 5d/w (inhalation)	not presented	*Experiment 1: shock motivated visual discrimination task. Experiment 2: food motivated spatial-discrimination task* DU: effect on errors (increased), no effect on relative rate of learning; UD: no effect; DD: effect on errors (increased), no effect on relative learning rate *Experiment 3: jump flinch study* DU: lowered jump and flinch thresholds UD: no effect; DD: lowered jump and flinch thresholds	Maternal toxicity not presented, No toxicity data on offspring
Koëter and Rodier (1986)	DUB/ICR mice (n=5-6/group; dams or litters)	a group of females on GD14 and another group of litters on PN2; Females exposed on GD14 were allowed to litter. Physical landmarks: • ear opening, PN5-7 • eye opening, PN15-17 Behavioural measures: • surface righting, PN6-10 • locomotion, PN8 and 15 • air righting, PN14-18 • general activity, PN20-23	0 and 0.5% halothane for 6h on GD14 and litters for 4h on PN2 (inhalation)	females exposed on GD 14 fell asleep during exposure.	• no effect on pup body weight • ear and eye opening retarded after prenatal exposure • air and surface righting were retarded during test period after pre- and postnatal exposure • locomotion was affected after pre- and postnatal exposure • total activity was not affected after pre- and postnatal exposure	After exposure on GD14 females resumed activity within a few minutes

Table 2.2 Developmental toxicity studies in animals with halothane.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Levin *et al*. (1986)	Sprague Dawley rats (F/litters exposed in experiment 1: 14 controls and 7 to 12.5 ppm to PN 30 experiment 2: 5 controls and 4 to 12.5 ppm to PN 30 experiment 3: 16 controls and 12 to 12.5 ppm to PN 30 and 12 to 12.5 ppm to PN60	females and their litter from GD2-PN30 or GD2-PN60 The study was divided in three experiments. Behavioural tests in male and female offspring	0 and 12.5 ppm 8h/d, 5d/w (inhalation)	Transient deficit on weight gain in pregnant females during gestation in experiment 3	A decreased pup weight PN23 and 32 was observed; on PN65 no differences in body weight were recorded (experiment 3). Impaired 8-arm maze performance in the GD2-PN30 and GD2-PN60 groups. Shock-escape deficit in the GD2-PN60 group. Effects were long.	
Levin *et al*. (1990)	Albino Norwegian rats (F/litters exposed in experiment 1 is 6-12 and in experiment 2 is 7-8)	Experiment 1: 0, 25 and 100 ppm, 24h/d, 7d/w from GD2-PN60 and 100 ppm , 8h/d, 5d/w from GD2-PN60 Experiment 2: 0 and 100 ppm 8h/d,5d/w from a. GD 2-PN30 b. PN 31-90 c. GD2-PN90 After 150 days testing on radial arm maze performance in male and female offspring behavioural tests in male and female offspring	see design (inhalation)	not presented	*Experiment 1:* decrease in radial arm performance in the all groups exposed to 100 ppm ; no effect in the 25 ppm group *Experiment 2:* performance deficit in the males of the PN31-90 group	Maternal toxicity data not presented No toxicity data on ofspring. In contrast to other studies, effects seems to be induced after postnatal exposure.

Table 2.3 Developmental toxicity studies in animals with halothane.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Chang *et al*. (1975a and b), Chang *et al*. (1976), Chang (1977), Dudley *et al*. (1977)	Sprague Dawley rat, 8 F/group	During gestation. Within 24 h after birth 4 randomly selected pups were killed and tissue samples of 1) the renal cortex were preserved 2) the liver were preserved 3) cerebral cortex. 100 days after birth 3 F1-animals were killed and tissue samples of 4) cerebral cortex preserved	0 and 10 ppm 8h/d, 5d/w during gestation (inhalation)	not presented	At electron microscopic examination Pups killed within 24h after birth: 1) Kidney:lesions in the proximal convoluted tubules 2) Liver: degenerative changes, leukocytic infiltration 3) Cerebral cortex: focal weakening and out-pouching of the nuclear envelope in many neurones, vacuolation of the Golgi complex of the neurons F1-animals killed after 100 days after birth 4) 10 ppm: residual changes in the neurones in the cerebral cortex	Maternal toxicity data not presented.
Mazze *et al*. (1986)	Sprague Dawley rat (F, control n=39-50, halothane n=18-24)	exposure on I. GD 14-16 II. GD 11-13 III. GD 8-10	0 and 0.8%, 6h/day (inhalation)	All exposed groups: light general anaesthesia Groups I and II slightly decreased growth Group III: statistically significantly decreased maternal growth	0.8% : group I and III, decreased foetal weight 0.8%: group I, increased number of developmental visceral variants (increased renal pelvic cavitation)	The increased number of visceral variants is most probably dus to the light maternal anaesthesia.

Annex **E**

Abbreviations

Abbreviations used:

body weight
day
female(s)
gestation day
hour
male(s)
number
part per million
postnatal
week

Conversion factor ppm to mg/m^3

8.21 mg/m^3