Enflurane

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

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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 Enfluraan. 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. 2002/12OSH, 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 enfluraan 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 enfluraan 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 enfluraan de ontwikkeling van het nageslacht niet schaadt. Zij adviseert daarom enfluraan niet te classificeren.
- Voor effecten tijdens lactatie, adviseert de commissie om enfluraan 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 enflurane.

The committee's recommendations are

- For effects on fertility, the committee recommends not to classify enflurane 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 enflurane 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 enflurane.

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 enflurane 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 labeling 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 Labeling 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 summarized 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 summarized 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

Enflurane

2.1 Introduction

Name	:	Enflurane
CAS-no	:	13838-16-9
Synonyms	:	2-chloro-1,1,2-trifluoroethyl difluoromethyl ether
Use	:	anaesthetic gas (since 1974)
Mol weight	:	184.49
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

Wyrobek *et al*. (1981) collected semen samples from 46 anaesthesiologists and 26 beginning residents in anaesthesiology and detected no differences in sperm concentration and number of abnormal sperm cells (Wyr81).

In a recent study, Peelen *et al*. (Pee99) reported that the time to pregnancy was not affected in operation chamber assistants (OR 0.9, 95% CI 0.6-1.4).

In both studies, however, 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 these studies are not sufficient for classification.

Samples of nasal ciliated epithelium were obtained from 18 non-smoking healthy patients, of which 8 were males. The samples were exposed for 3 hours to either 5% enflurane, 3.6% isoflurane or 2.25% halothane. Over a 4 hour observation period the cilia beat frequency reduced significantly compared with controls exposed to air alone. The effects of the three compounds was similar (Rap96a). After 1 hour exposure period the beat frequency returned to baseline values after 60 minutes of washout of enflurane and isoflurane (Rap96b). Studies on the effect on spermatozoa motility were not available.

Development

Occupational exposure

Several epidemiological studies were performed in which females occupationally exposed to anaesthetics and wives of males occupationally exposed to anaesthetics were inquired about the course and outcome of their pregnancies, with specific attention for miscarriages and congenital anomalies (Ame74, Cor74, Pha77, Ros78, Eri79, Lau81, Hem85, Joh87, Gui90, Mat93). In all studies, except Eri79, Lau81, Hem 85 and Joh87, some effects on these parameters were suggested. However, most studies were criticized by several authors (Fer78, Ves78, Dud81, Tan85) 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 enflurane was one component, the duration and level of exposure and its timing in pregnancy and other exposure were often not reported. Most of these objections were also true for the more recent studies (Hem85, Joh87, Gui90, Mat93).

In a recent study, Peelen *et al*. (Pee99) found no increased risk for spontaneous abortion, preterm birth and congenital abnormalities among operation personal after correction for alcohol use, work circumstances and other occupational exposure. Women present at the beginning of operations had higher risks for preterm birth and women present at tonsil operations for spontaneous abortions. In this study operation personnel was exposed to a mixture of anaesthetic gasses and duration of exposure was

not specified. For that reasons it was not clear if enflurane caused the slight increases in reproductive effects.

The committee concluded that none of the studies available could be used for the classification of enflurane for the aforementioned reasons.

Exposure of pregnant woman during surgery or delivery

Several studies have been performed regarding anaesthetics during pregnancy or at delivery and pregnancy outcome. Stefani *et al*. (Ste82) found no differences in neurobehaviour within 24 h after birth between infants born of mothers anaesthetized with enflurane alone (0.3-0.8% [22650-60400 mg/m^3]) during parturition and born of mothers not anaesthetized during parturition.

In a cohort study in Canada among women who underwent surgery with anaesthesia and women who underwent no surgery during pregnancy, no association was found between the incidence of anomalies and anaesthesia nor between the rate of abortion and anaesthesia during pregnancy (Dun86). However, taking the type of anaesthesia into account (general, local, spinal/block), a significant increase in abortion rate was found in women who underwent general anaesthesia during pregnancy (RR 1.58, 95% CI 1.19-2.09). In this study, no correction was made for socio-economic and life-style factors. Furthermore, the relative effect of the anaesthesia versus other variables (eg site of operation, indication for the procedure, co-medication) is unclear.

A case-control study with 694 infants with major central nervous system defects, showed no association between the total of these effects or between single effects and anaesthesia undergone by their mothers during pregnancy (Syl94). However, hydrocephalus with any other effect (OR 2.9, 95% CI 1.2, 6.8) and especially with eye defects (OR 39.6, 95% CI 7.5, 209.2) was found to be associated with anaesthesia during pregnancy.

In both studies the anaesthetic gas used was not specified. Therefore, it is not clear if enflurane caused the increases in developmental effects.

Lactation

No publications were available concerning the excretion of enflurane in human breast milk.

2.3 Animal studies

Fertility and developmental toxicity studies with enflurane in experimental animals are summarized in tables 3 and 4 (Annex D), respectively.

Fertility studies

Male (C57B1/C3H)F1 mice were exposed by inhalation to air, 0.12 and 1.2% [9060 and 90600 mg/m^3] enflurane for 4 h/day for 5 days (Lan81). After 28 days epididymal spermatozoa were evaluated for morphological changes. The percentage abnormal spermatozoa of the animals exposed to the highest concentration of enflurane was slightly increased compared to the control (2.04% ± 0.13 vs. 1.42% ± 0.08). For the lowest concentration group no difference in abnormal spermatozoa was observed. Twenty percent of the animals in the high-dose group died; other toxic effects were not described in this study. The committee doubts the relevance of these findings for the (human) fertility.

Wharton *et al*. (Wha81) exposed 4 week old male Swiss/ICR mice during 11 weeks prior to mating with non-exposed females inhalatory to 0, 0.01, 0.1 and 1% [755, 7550 and 75500 mg/m^3] enflurane for 4 h/day 5 days/week. The males showed reduced weight gain after 5.5 weeks of exposure. Therefore the highest concentration was reduced to 0.5% [37750 mg/m^3]. No difference in pregnancy rate, number of implantations, live foetuses or reproduction loss was observed.

Similar parameters (on number of implantations, live foetuses or reproduction loss) were also unaffected after inhalatory exposure of male Sprague Dawley rats to 20 and 200 ppm [151 and 1510 mg/m^3] enflurane during 63 days prior to mating with non-exposed females for 8h/day 5 days/week (Hal81, Gre82). Paternal toxic effects were not observed.

Cameron *et al*. (Cam83) did not find any influence on serum testosterone, luteinizing hormone and follicle stimulating hormone in male Sprague-Dawley rats after inhalatory exposure to 50, 500 and 1000 ppm [377.5, 3775 and 7550 mg/m^3] enflurane 6h/day for 11, 9 and 3 days, respectively. General toxic effects were not presented.

Developmental toxicity

Saito *et al*. (Sai74) exposed pregnant ddY mice from gestation day (GD) 7-12 during 1 h/day inhalatory to 0, 0.05 and 0.75% [3775 and 56625 mg/m^3] enflurane and pregnant Wistar rats from GD 9-14 during 1 h/day inhalatory to 0, 0.05 and 1.25% [3775 and 94375 mg/m^3] enflurane. Fifteen rats and mice underwent Caesarean section whereas 5 animals were allowed to deliver. No treatment related differences on a range of developmental features, including external, visceral, skeletal and behavioural development were found in any of the species. Treatment related differences in maternal body weight were not observed.

Strout *et al*. (Str77) treated pregnant Sprague Dawley rats from GD 1-18 inhalatory with 0, 10.7 and 63.7 ppm [80.8 and 480.9 mg/m^3] enflurane for 8 h/day. Litter size was not affected, but birth weights of exposed offspring were higher than in controls. Cross-fostering studies showed no effects on body weight after exposure to the low-dose. In the high-dose groups, offspring delivered by untreated and cross-fostered by treated dams or vice versa showed decreased body weight at PN 7, but not at PN 14 or 21. Offspring delivered and fostered by treated dams showed an increased body weight at PN 21. No maternal toxicity was observed. The committee is of the opinion that the effects on body weight were minor effects and might be due to the modest anaesthetic effect of enflurane.

Pope and Persaud (Pop78) observed lower foetal weights after inhalatory exposure of Sprague Dawley rats on GD 0-20 to 3200 ppm [24160 mg/m^3] enflurane for 8h/day in the absence of maternal toxicity.

Wharton *et al*. (Wha81) treated in a first experiment female Swiss/ICR mice inhalatory with 0, 0.01, 0.1 and 1% [755, 7550 and 75500 mg/m^3] enflurane 4h/day, 7 days/week. The exposure started 3 weeks prior to mating with untreated males and was continued until GD 18. The highest dose was decreased to 0.5% [37750 mg/m^3] at the beginning of the mating period as females showed reduced weight gain during the premating period. No difference in gestational weight was observed. A dose related increase was seen in lumbar rib formation reaching statistical significance in the highest treatment group. Also, a higher incidence of increased renal pelvic cavitation was observed in this group. No effect was found on the number of live foetuses and implantations, percentage of resorptions or foetal death and foetal weight. In a following experiment, exposure of female mice to aforementioned concentrations levels during GD 6-15 resulted in a decrease in foetal weight, length and ossification and an increased incidence of major malformations (cleft palate), minor skeletal anomalies and variants, visceral variants (increased renal pelvic cavitation) and minor anomalies (enlarged brain ventricle) at the highest concentration. At this concentration (1%) a reduced gestational weight was observed. Treatment at the same concentrations levels of males 11 weeks prior to mating with untreated females showed no difference in foetal weight and length.

Chalon *et al*. (Cha81) studied the maze performance of albino mice 6-7 weeks of age exposed during pregnancy by inhalation to 2 and 4% [151000 and 302000 mg/m^3] enflurane on GD 6 and 11 or 14 and 17 during 30 minutes. Treated mice were slower on day 3, 5, 7 and 10 of the training period (10 days) compared to controls, but not on the first day. Maternal toxicity was not presented.

Exposure of male Sprague Dawley rats to 20 and 200 ppm [151 and 1510 mg/m^3] enflurane 8h/day for 5 days/week during 63 days prior to mating with non-treated females did not result in any treatment related differences in foetal and placental

features compared to controls (Hal81, Gre82). Similarly, no effects were found in the offspring of females exposed to the same concentrations of enflurane 28 days prior to mating with non-treated males and during gestation. No effects on fertility and reproduction parameters were detected among the progeny of offspring of males exposed inhalatory to 20 ppm enflurane during 63 days prior to mating with non-treated females (Hal81). At 200 ppm these effects were not studied (Gre82). General toxic effects were not observed.

Fischer 344 rats exposed in utero to 1500 ppm [11325 mg/m^3] enflurane on GD 0-20 during 6h/day did not show any treatment related differences in a battery of behavioural tests at several ages (Pet82). Differences found were a shortened pentobarbital sleep time at puberty indicative of a changed metabolism and an increased percentage of litters with 6 or less pups (35% versus 5% in controls). Detailed litter data were not given. Mean pup weight and postnatal growth were unaffected. Effects on maternal weight gain and behaviour were not observed.

Mazze *et al*. (Maz86) exposed pregnant Sprague Dawley rats by inhalation to 1.65% [124575 mg/m^3] enflurane for 5 h/day on GD 14-16 (period I), for 6h/day on GD 11-13 (period II), or 6 h/day on GD 8-10 (period III). A decreased foetal weight was observed in the period I and period III treated groups. When exposed from GD 11-13 (period II) an increased incidence of rudimentary lumbar rib was observed. All dams suffered from light general anaesthesia, whereas the dams exposed GD 14-16 (period I) showed lower weight gain. No effects were found on number of implantations/dam, live foetuses/dam and resorptions/dam.

Lactation

No publications were available.

2.4 Conclusion

Only two studies concerning the effects of anaesthetic gasses on human fertility were available (Wyr81, Pee99). In these studies no effects on sperm quality (Wyr81) and time to pregnancy (Pee99) were observed. However, no conclusions can be drawn about the effects of enflurane on human fertility, as the anaesthetic gasses used were not specified. For this reason, the committee is of the opinion that a lack of appropriate human data preclude the assessment of enflurane for effects on fertility.

In male mice and rats exposed inhalatory to 337.5-37750 mg/m^3 enflurane no changes in pregnancy rate, number of implantations, live foetuses, reproduction loss and hormone levels were observed (Wha81, Hal81, Gre82, Cam83). The increase in abnormal sperm morphology in mice found by Land *et al*. in the high dose group was

only slight and in addition mortality was observed (Lan81). No studies concerning female fertility were available.

In view of these animal data, a lack of appropriate data precludes the assessment for fertility.

Epidemiological studies considering occupational exposure (Ame74, Cor74, Pha77, Ros78, Eri79, Lau81, Hem85, Joh87, Gui90, Mat93, Pee99) gave rise to concern about the effects of anaesthetic gas mixtures on abortion, foetal development, preterm birth and congenital anomalies. In addition, a cohort study regarding anaesthesia during pregnancy and pregnancy outcome showed general anaesthesia to be associated with an increase in abortion rate (Dun86). In a case control study, hydrocephalus with any other congenital effect, and especially with eye defects, was associated with anaesthesia during pregnancy (Syl94). However, as the anaesthetic gasses used were not specified in any of these studies, the committee is of the opinion that it is not clear whether the effects described were caused by enflurane. A study comparing infants born of mothers under 0.3-0.8% [22650-60400 mg/m^3] (pure) enflurane anaesthesia alone and infants of mothers born without anaesthesia showed no differences in neurobehaviour 2 to 24h after parturition (Ste82). Considering the above described studies, the committee is of the opinion that a lack of appropriate human data preclude the assessment of enflurane for effects on development.

In several studies in rats and mice, effects of enflurane on development were observed. Pope and Persaud (Pop78) and Mazze *et al*. (Maz86) found a decreased foetal weight after exposure of female Sprague Dawley rats to 3200 ppm [24160 mg/m^3] and 1.65% enflurane [124575 mg/m^3], respectively. In addition, an increased incidence of rudimentary lumbar rib was found in the latter study. However, the committee considers these findings as secondary to growth retardation. In addition, the effects were found in the presence maternal toxicity (light anaesthesia) (Maz86) or maternal toxicity could be expected based on the concentration used (Pop78). Wharton *et al* (Wha81) found an increase in lumbar rib formation as well as a higher incidence of increased renal pelvic cavitation in foetuses of Swiss/ICR mice treated 3 weeks prior to mating with untreated males with 1% [75500 mg/m^3] and during gestation with 0.5% [37750 mg/m^3]. The high concentration during premating resulted in lower body weight gain, whereas during gestation no maternal toxicity was observed. Exposure of pregnant mice to 1% [75500 mg/m^3] during gestation resulted in a decrease in foetal weight, length and ossification and a variety of skeletal and visceral anomalies, malformations and variants. However, a reduced gestational weight was observed at this concentration. Chalon *et al*. (Cha81) found 6 to 7 week old mice exposed in utero to 2 or 4% [151000 or 302000 mg/m^3] enflurane to perform slower in the maze than control mice. In this study, maternal toxicity was not described. The committee

considers that the doses used in Wharton *et al* (Wha81) and Chalon *et al* (Cha81) are likely to have caused maternal effects.

In view of the animal studies with respect to the effects on development, the committee concludes that only effects on foetal body weight were found in the presence of maternal toxicity (light anaesthesia). Therefore, the committee is of the opinion that sufficient animal data show that no classification of enflurane is indicated.

As no studies on enflurane in breast milk were available, the committee recommends not to label enflurane for effects during lactation because of a lack of appropriate data.

Proposed classification for fertility

A lack of appropriate human and animal data precludes the assessment of enflurane for effects on fertility.

Proposed classification for developmental toxicity

A lack of appropriate human data precludes the assessment of enflurane for effects on development and sufficient animal data show that no classification is indicated.

Proposed labeling for effect during lactation

Lack of appropriate data precludes the assessment of enflurane 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 organizations have commented on the draft review:

- 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

See next pages.

Table 1.1 Fertility studies in man.

authors	exposure	study type/data collection	study/comparation population	investigated effects and results	remarks
Wyr81	Mixture: waste anaesthetics Exposure for at least one year	Cohort study/ semen sampling	46 anaesthesiologist 26 beginning residents in anaesthesiology	No effect on sperm concentration and sperm abnormalities	Mixed exposure Controlled for smoking, medical history, sauna use
Pee99	Mixture: waste anaesthetics 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

Tabel 2.1 Developmental toxicity studies in man.

authors	exposure	study type/data collection	study/comparation population	investigated effects and results	remarks
Ame74	Mixture: waste anaesthetics *Females*: exposure during 1st trimester of pregnancy and work in OT during previous calendar year. *Males*, work in OT during year prior ro pregnancy	Retrospective survey USA 1972-1974/postal questionnaire	18,568 pregnancies in 29,810 exposed OT personnel from 4 societies 5,620 pregnancies in 10,420 unexposed physicians, nurses and their wives from 2 societies plus unexposed individuals and their wives from study population	Increase of spontaneous abortions in exposed females Increase of congenital abnormalities in exposed females and wives of exposed males	Mixed exposure Rates were standardised by the direct method adjusted for maternal age and smoking
Cor74	Mixture: waste anaesthetics OT employment during pregnancy	Retrospective survey USA / postal questionnaire and telephone interview	434 births to 268 nurses who practised anaesthesia during pregnancy 261 births to nurses who did not practice anaesthesia during pregnancy and published incidence rates	Increase in congenital abnormalities	Mixed exposure Age was not a factor in the observed differences between exposed and non-exposed groups
Pha77	Mixture: waste anaesthetics Appointment as anaesthesiologist at time of conception	Retrospective survey UK/ postal questionnaire	670 pregnancies while employed as anaesthesiologist 1,977 pregnancies while women had no medical appointments	Risk on spontaneous abortion, low birth weight, stillbirths and congenital abnormalities (cardiovascular) increased	Mixed exposure Effects were standardised for maternal age, smoking habits and parity
Ros78	Mixture: waste anaesthetics Member Finnish Society of Anaesthesiologists	Retrospective survey Finland 1961-1976/postal questionnaire	248 pregnancies in anaesthesiologists families 266 pregnancies in pediatricians families (no OT exposure)	No difference in spontaneous abortions Low birth weight and congenital abnormalities (musculoskeletal) increased	Mixed exposure Results were corrected for smoking habits

Table 2.2 Developmental toxicity studies in man.

authors	exposure	study type/data collection	study/comparation population	investigated effects and results	remarks
Eri79	Mixture: waste anaestheticswomen working in OT during pregnancy who gave birth in 1973 and 1975	Cohort Sweden 1973 and 1975/Registry data	494 women who worked throughout pregnancy, 37 women who worked more than half of their pregnancies and 10 women who worked less than half of their pregnancies 19,127 women employed in medical work who delivered in 1973 or 1975	No difference in birth weight, perinatal death rate, congenital abnormalities Pregnancy duration in weeks decreased	Mixed exposure Corrections were made for maternal age and parity
Lau81	Mixture: waste anaesthetics Exposure anaesthetic gasses by one or both parents during or in the year before pregnancy	Retrospective survey Belgium/ postal questionnaire	Pregnancies in 149 anaesthesiologists and their wives and 240 OT nurses and their wives/pregnancies in 531 occupational physicians, dermatologists, intensive care, and other nurses and their wives	No difference in spontaneous abortions, sum of all abnormal pregnancies, premature birth, stillbirth and congenital abnormalities Increased number of males born	Mixed exposure No differences in maternal smoking habits between different groups
Ste85	No inhalation agent (n=21), 0.3-0.8%[22650 and 60400 mg/m3] enflurane and oxygen (n=22)	Experimental design	Analgesia during vaginal delivery of baby, infants underwent neurobehavioural testing*	No significant differences in neuro behavioural status occurred	*Neurologic and Adaptive Capacity Score at 15 min, 2 and 24 h and Early Neonatal Neurobehavioural Scale at 2 and 24h
Hem85	Mixture: waste anaesthetics exposure during 1st trimester	Case-control Finland 1973-1979/ registry data for outcomes postal questionnaire	1.169 employed nurses who had spontaneous abortion/ 469 employed nurses who gave birth to a healthy infant 2. 38 employed nurses who gave birth to an infant with congenital abnormalities/ 99 employed nurses who gave birth to a healthy infant 1 and 2: cases excluded from controls	No significant increase in risk of spontaneous abortion or congenital abnormalities	1 and 2. Mixed exposureMatched on maternal age and other potential exposures

Table 2.3 Developmental toxicity studies in man.

authors	exposure	study type/data collection	study/comparation population	investigated effects and results	remarks
Dun86	Mixture: anaesthesia during pregnancy	Cohort Canada 1971-1978 health insurance data and provincial congenital-anomalies registry	Women who underwent surgery with anaesthesia during pregnancy and women who did not (n=2565) women were matched on geographic area and age groups were compared for abortion rate and frequency of anomalies	No association was found between the incidence of anomalies or between the rate of abortion and anaesthesia during surgery by subdividing in type of anaesthesia (general, local, spinal/block) a significant increase in abortion rate was found in those women who underwent general anaesthesia (RR 1.58, 95% CI 1.19-2.09) when compared with their matched controls	Anaesthetic gas used was not specified Socio-economic and lifestyle factors were not taken into account
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 exposureResults 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 exposureOR's were standardised for age, smoking, alcohol consumption, previous abortion and occupation

Table 2.4 Developmental toxicity studies in man.

authors	exposure	study type/data collection	study/comparation population	investigated effects and results	remarks
Mat93	Mixture: waste anaesthetics	Case-control USA 1968-1980/ telephone questionnaire, cases were registered with the Metropolitan Atlanta Congenital Defects Program	4915 cases (live and still-born infants with serious malformations) and 3027 control babies	Potential maternal exposure to anaesthetic gasses was associated with spina bifida (RR = 6.27; 95% CI 1.54-25.48) offspring of fathers exposed to anaesthetic gasses had a significantly decreased risk of birth defects (RR = 0.45; 95% CI 0.21-0.98)	Mixed exposure RR for maternal exposure based on only three cases No adjustment was made for confounding factors, however, when adjustment was applied for maternal education, age and alcohol consumption, results were the same
Syl94	Mixture: anaesthesia during surgery in first trimester of pregnancy	Atlanta birth defects case-control study 1968-1980/ telephone interviews	694 infants with major CNS defects, Metropolitan Atlanta Congenital Defects Program 2984 controls, 1% random sample Atlanta metropolitan	No relation with anaesthesia was observed taking all CNS defects into account. No significant associations were found between isolated effects and anaesthesia. Hydrocephalus with any other effect (OR 2.9, 95% CI 1.2, 6.8) and especially with eye defects (OR 39.6, 95% CI 7.5, 209.2) was found to be associated with general anaesthesia	Anaesthetic gas used was not specified Age, median parity, smoking and drinking, mean weight gain and mean education were equal for both groups Odds ratios were adjusted for race, hospital of birth and period of birth
Pee99	Mixture: waste anaesthetics 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 (OR 1.3, 95% CI 0.8-2.1), preterm birth (OR* 1.4, 95% CI 0.7-2.8) and congenital abnormalities (OR* 1.8, 95% CI 1.0-4.1)	Mixed exposure *Controlled for age, education, menstrual cycle, life style and circumstances during work.

Table 3.1 Fertility studies in animals with enflurane.

authors	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs and reproduction	remarks
Lan81	Male (C57B1/C3H)F1 mice (low n=5, high n=10)	4h/day during 5 consecutive weekdays sacrifice 28 days after start of exposure sperm morphology of both cauda epididymides	0, 0.12 and 1.2% [9060 and 90600 mg/m^{3}] enflurane inhalation	At high concentration 2 animals died. paternal toxicity not described	At high concentration percentage of abnormal spermatozoa increased	
Wha81	Male Swiss/ICR mice (n=10)	11 weeks premating 4h/day, 5 days/week mating with untreated females	0, 0.01, 0.1 and 1% [755, 7550 and 75500 mg/m^{3}] enflurane inhalatory	Reduced weight gain of males exposed at highest concentration* No difference in gestational weight gain	No difference in pregnancy rate, number of implantations, live foetuses and reproductive loss	*highest dose was reduced to 0.5% after 5.5 weeks of treatment as males were failing to gain weight
Hal81	Male Sprague Dawley rats (n=20)	8h/day, 5 consecutive days/week for 99 days after 63 days males were mated with untreated females	0 and 20 ppm [151 mg/m^{3}] enflurane inhalatory	No effect on body weight gain, liver and kidney weight or histology	No differences in pregnancy rates, number of live foetuses or implantations and percentage resorptions or dead in utero	
Gre82	Male Sprague Dawley rats (n=20)	8h/day, 5 consecutive days/week for 100 days after 63 days males were mated with untreated females	0 and 200 ppm [1510 mg/m^{3}] enflurane inhalatory	No effect on body weight gain, liver and kidney weight or histology	No differences in pregnancy rates, number of live foetuses or implantations and percentage resorptions, deciduomata or dead in utero	
Cam83	Male Sprague Dawley rats (n=5)	6h/day for 3, 9 or 11 days at high, mid and low concentration	0, 50, 500 and 1000 ppm [377.5, 3775 and 37550 mg/m^{3}] enflurane inhalatory	No effects presented	No influence on testosterone, LH and FSH levels observed	

Table 4.1 Developmental toxicity studies in animals with enflurane.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Sai74	Pregnant ddY mice (n=20)	Exposure from GD 7 until GD 12 during 1 h/day aesarean section at day GD 18 (n=15) or allowed to deliver naturally (n=5)	0, 0.05% and 0.75% [3775 and 56625 mg/m^3] inhalatory	No differences in maternal body weight	No differences in number of implantations, number of dead foetuses, foetal body weight, sex ratio, external, internal and bone anomalies no treatment related differences in percentage live births and survival at 3 and 6 weeks of age, pup body weights, external, auditory and behavioural features at 3 weeks and anatomical, sex and bone features at 6 weeks of age	
Sai74	Pregnant Wistar rats (n=20)	Exposure from GD 9 until GD 14 during 1 h/day aesarean section at day GD 18 (n=15) or allowed to deliver naturally (n=5)	0, 0.05% and 1.25% [3775 and 94375 mg/m^3] inhalatory	No differences in maternal body weight	No differences in number of implantations, number of dead foetuses, foetal body weight, sex ratio, external, internal and bone anomalies no treatment related differences in percentage live births and survival at 3 and 6 weeks of age, pup body weights, external, auditory and behavioural features at 3 weeks and anatomical, sex and bone features at 6 weeks of age	
Str77	Pregnant Sprague Dawley rats (n=6-13)	Exposure from GD 0 to GD18 for 8 h/day after delivery cross-fostering study	0, 10.7 and 63.7 ppm [80.8 and 480.9 mg/m^3] inhalatory	No differences in weight or weight gain, feeding habits, behaviour or appearance	No significant difference in litter size birth weight of both treated groups significantly increased no weight differences during lactation among offspring of the low-dose group in the high-dose group offspring delivered by control mothers and cross-fostered by treated mothers and offspring of treated mothers fostered by untreated mothers had a lower weight at PN 7, but not at PN 14 or PN 21, offspring of treated mothers fostered by treated mothers had higher birth weights at PN 21	
Pop78	Pregnant Sprague Dawley rats (n=5-8)	Exposure from GD 0 to GD 20 for 8 h/day	0 and 3200 ppm [24800 mg/m^3] inhalatory	No maternal deaths no significant changes in weight gain no pathological changes	Significant decrease in mean body weight of foetuses of exposed mothers compared to controls, no increase in the incidence of foetal resorptions all foetuses at term were alive and showed no external defects or major skeletal effects no pathological changes	

Table 4.2 Developmenal toxicity studies in animals with enflurane.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Wha81	Female Swiss/ICR mice (n=39-48)	3 weeks prior to mating continuously until GD 18, 4h/day, 7 days/week mating with untreated males	0, 0.01, 0.1 and 1% [755, 7550 and 75500 mg/m^3] enflurane inhalatory	Highest concentration induced ataxia followed by light sleep and reduced weight gain* no difference in gestational weight gain	No differences in pregnancy rates, number of implantations and live foetuses, percentage of resorptions or foetuses dead in utero and mean foetal weight dose-related increase in lumbar rib formation, significant at highest concentration increased incidence of IRPC at highest concentration	*highest dose was reduced to 0.5% at the beginning of the mating period as females were failing to gain weight
Wha81	Pregnant female Swiss/ICR mice (n=26-34)	GD 6-15, 4h/day, 7 days/week	0, 0.01, 0.1 and 1% [755, 7550 and 75500 mg/m^3] enflurane inhalatory	Reduced gestational weight gain at highest concentration	No differences in pregnancy rates, number of implantations and live foetuses, percentage of resorptions or foetuses dead in utero at highest concentration mean foetal weight and length and ossification decreased and increased incidence of major malformations (mostly cleft palate), minor skeletal anomalies (bent and fused ribs, fused vertebrae), skeletal variants (mostly lumbar ribs), visceral variants (mostly IRPC) and minor visceral anomalies	
Wha81	male Swiss/ICR mice (n=10)	11 weeks premating 4h/day, 5 days/week mating with untreated females	0, 0.01, 0.1 and 1% [755, 7550 and 75500 mg/m^3] enflurane inhalatory	Reduced weight gain of males exposed at highest concentration*no difference in gestational weight gain	No difference in foetal weight and foetal length	*highest dose was reduced to 0.5% after 5.5 weeks of premating treatment as males were failing to gain weight

Table 4.3 Developmental toxicity studies in animals with enflurance.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Cha81	Albino mice (n=6), aged 6-7 weeks	mothers of mice were exposed on GD6 and 11 or GD 14 and 17 for 30 minutes mice were trained 5 times on maze performance (day 1, 3, 5, 7 and 10)	0, 2 and 4% [151000 and 302000 mg/m^3] enflurane inhalatory	Not presented	All mice born from exposed mothers were slower in maze performance from day 3-10 of training than control mice	
Hal81	Male Sprague Dawley rats (n=20)	8h/day, 5 consecutive days/week for 99 days after 63 days males were mated with untreated females progeny was mated	0 and 20 ppm [151 mg/m^3] enflurane inhalatory	No effect on body weight gain, liver and kidney weight or histology	No difference in placental weight, crown rump length or sex ratio foetal weight slightly decreased, probably as litter size was larger no difference in skeletal abnormalities no fertility or reproduction effects in first generation	
Hal81	Female Sprague Dawley rats (n=20)	8h/day, 5 consecutive days/week for 28 days prior to mating and during gestation after 28 days females were mated with untreated males	0 and 20 ppm [151 mg/m^3] enflurane inhalatory	No effect on body weight gain and liver and kidney weight or histology	No differences in pregnancy rates, number of live foetuses or implantations and percentage total foetal loss no difference in placental weight, crown rump length or sex ratio foetal weight slightly decreased due to larger litter size no difference in skeletal abnormalities	
Gre82	Male Sprague Dawley rats (n=20)	8h/day, 5 consecutive days/week for 100 days after 63 days males were mated with untreated females	0 and 200 ppm [1510 mg/m^3] enflurane inhalatory	No effect on body weight gain, liver and kidney weight or histology	No difference in foetal and placental weight, crown rump length or sex ratio significant less skeletal abnormalities after treatment with enflurane*	

Table 4.4 Developmental toxicity studies in animals with enflurane.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Gre82	Female Sprague Dawley rats (n=20)	8h/day, 5 consecutive days/week for 28 days prior to mating and during gestation after 28 days females were mated with untreated males	0 and 200 ppm [1510 mg/m^3] enflurane inhalatory	No effect on body weight gain and liver weight or liver and kidney histology kidneys slightly enlarged	No differences in pregnancy rates, number of live foetuses or implantations and percentage resorptions, deciduomata or dead in utero no difference in foetal and placental weight, crown rump length or sex ratio increase in supplementary ribs after treatment with enflurane*	*Controls values were low compared to other control values, all values within normal range therefore no treatment related increase
Pet82	Pregnant Fischer 344 rats (n=30)	6h/day during GD 0-20 mothers were allowed to litter and raise their young until PN 28	0 and 1500 ppm [11325 mg/m^3] enflurane inhalatory	No effect on maternal weight gain or gross behaviour	Exposed females had 35% small litters (6 pups) whereas controls 5% no difference in pup weight, weight gain and postnatal survival through 75 weeks no difference in righting reflex, temperature regulation and eye opening or organ*/body weights at any age no differences in clinical chemical or haematological profile behaviour tests** performed at several ages did not show differences at puberty pentobarbital sleep time was significantly shortened after exposure in males but not at adult and geriatric age and not in females	*brain, pituitary, thyroid, thymus, lungs, heart, kidney, adrenal, liver, spleen, prostate, testis, epididymis, seminal vesicles, ovary and uterus **righting reflex, inclined screen, open-field activity, food maze behaviour, activity wheel, swimming stress test, shock avoidance learning
Maz86	Pregant Sprague Dawley rats (n=39-50 control, 20-23 enflurane)	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 1.65% [124575 mg/m^3] enflurane inhalation	I, II and III: light anaesthesiaI : decreased weight gain	II: increased number of developmental variants (rudimentary lumbar rib)	

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