Nitrous oxide

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 reprotox-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 lachgas. 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

Nitrous oxide

Evaluation of the effects on reproduction, recommendation for classification

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

to

the Minister and State Secretary of Social Affairs and Employment

No. 2000/03OSH, The Hague, 1 May 2000

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Contents

Samenvatting

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

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit adviseert de commissie lachgas in categorie 3 (*stoffen die in verband met hun mogelijke voor de vruchtbaarheid van de mens schadelijke effecten reden geven tot bezorgdheid*) te classificeren en met R62 (*mogelijk gevaar voor verminderde vruchtbaarheid*) te kenmerken.
- Voor effecten op de ontwikkeling adviseert de commissie lachgas 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 lactatie adviseert de commissie om lachgas *niet* te kenmerken wegens onvoldoende gegevens.

Executive summary

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

The committee's recommendations are:

- For effects on fertility, the committee recommends to classify nitrous oxide in category 3 (*substances which cause concern for human fertility*) and to label nitrous oxide with R62 (*possible risk for impaired fertility*).
- For developmental toxicity, the committee recommends to classify nitrous oxide in category 3 (*substances which cause concern for humans owing to possible developmental toxic effects*) and to label nitrous oxide 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 nitrous oxide 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 nitrous oxide by the Health Council's Committee for Compounds Toxic to Reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by Mrs ir 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 and development and lactation of the above mentioned compound.

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

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

In November 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, e.g. 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 1998. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1998 to 1999. The results of this search were no reason for the committee to adjust the recommendations.

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

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

1.6 Presentation of conclusions

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

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

1.7 Final remark

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

* for definitions see Tox95

Chapter 2

Nitrous oxide

2.1 Introduction

Name	:	nitrous oxide
CAS No.	:	10024-97-2
Use	:	anaesthetic gas
Mol weight	:	44.01
Chem formula	:	N_2O
Conversion factors	:	1% = 10.000 ppm = 18 g/m^3
		1 ppm = 1.8 mg/m^3

2.2 Human studies

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

Fertility

Wyrobeck *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 (Wyr81).

Anderson *et al*. (1992) found no differences in the quality of sperm of surgical patients before and after exposure to N_2O in combination with halothane (And92).

Knill-Jones *et al*. (1972) reported unexplained infertility amongst female and male anaesthetists (Kni72). In 1975, Knill-Jones *et al* detected no effects of paternal exposure to a mixture of anaesthetics on involuntary infertility in a case-control study (Kni75).

Rowland *et al*. (1992) reported effects on time to pregnancy and spontaneous abortion in female dental assistants after unscavenged exposure to N_2O (Row92).

Ahlborg *et al*. (1996) reported an effect of N_2O on time to pregnancy in a small group of midwives who assisted in more than 30 deliveries (N_2O exposed) per month (Ahl96).

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 several of anaesthetic gasses were measured; the maximal N_2O concentration measured was 318 mg/m^3 (0.02%).

In these human studies, 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 N_2O was one component, the duration of exposure and its timing in pregnancy and exposure to other chemicals such as inorganic mercury (amalgam), were often not reported.

For these reasons, the committee considered the quality of these studies to be insukficient for classification.

Development

Several epidemiological studies were performed in which female anaesthetists, dental assistants, 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, Kni72, Ros73 Ame74, Cor74a, Coh75, Pha77, Ros78, Eri79, Coh80, Lau81, Hei84, Hem85, Joh87, Row92). In all studies, except Eri79, Lau81, Hei84 and Hem 85, some effects on these parameters were suggested. 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 groups and no consideration was given to social factors, medication, illnesses and possible stress.

Furthermore, the composition of the anaesthetic gas mixtures, in which N_2O was one component, the duration of exposure and its timing in pregnancy and other exposure such as inorganic mercury (amalgam), were often not reported.

For these reasons, the committee considered the quality of these studies to be insukficient for classification.

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 (Pee 99). An increased risk for spontaneous abortion (OR, odds ratio =1.3 (CI 95%: 0.8-2.1)), preterm birth (OR=1.9 (CI 95%: 1.2-3.0)) and congenital abnormalities (CI 95%: OR=1.6 (0.9-2.9)) 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 N_2O concentration measured was 318 mg/m^3. However, operation personnel was exposed to a mixture of anaesthetic gasses. For that reason it was not clear if N_2O caused the slight increase in reproductive effects.

Lactation

No publications were found concerning the excretion of nitrous oxide in human breast milk.

2.3 Animal studies

Fertility and developmental toxicity studies with N_2O in experimental animals are summarised in annex D (table 3 and 4), respectively.

Fertility

Kripke *et al*. (1976) reported a significant reduction in mean testicular weight and damage to dividing spermatogenic cells in the seminiferous tubules in all rats (LEW/f Mai) exposed to a mixture of 20% N_2O (360 g/m^3), 20 % oxygen and 60% nitrogen for various time periods up to 35 days (Kri76). Evidence of injury of the seminiferous tubules was found in some animals after exposure for 2 days; by 14 days such damage was found in all animals. Recovery of spermatogenesis was observed after removing the animals to room air for 6 days. However, repair of the histological architecture of affected tubules was observed in only 1 of 5 animals sacrificed after 6 days in room air. Other animals had still severely affected tubules 10 days after removal from N_2O-exposure. General toxicity was not described in this study.

Male (C57B1/C3H)F1 mice were exposed by inhalation to air, containing 8 or 80 % N_2O (144 or 1440 g/m^3) for 4 h/day for 5 days (Lan81a). After 28 days, epididymal sperm was evaluated for morphological changes. The percentage abnormal sperm in both N_2O-exposed groups was comparable to the control group (1.42 $\pm$ 0.08 (8%

group), 1.64 ± 0.15 (80% group) versus 1.44 ± 0.19 in the control). General toxicity was not described in this study.

Male mice (Swiss/ICR) were exposed to 0, 0.5, 5 or 50% N_2O (0, 9, 90 or 900 g/m^3) for 4 h/day, 5 days/week for 9 weeks and then mated overnight for 1 week to untreated, virgin females (Maz82). No effects on fertility were observed. General toxicity was not described in this study.

Male and female Swiss Webster mice were exposed to 0, 0.5, 5 or 50% N_2O (0, 9, 90 or 900 g/m^3) by inhalation for 4 h/day, 5 days/week for 14 weeks (Maz83). After exposure, germ cells were examined for evidence of injury. There were no significant differences among the 4 inhalation exposure groups in testes weight, percentage of abnormally shaped sperm, sperm count or histological appearance of the testes; the mean percentage (± SE) of abnormal sperm cells ranged from 8.9 ± 2.4 (5% N_2O) to 13.5 ± 0.5 (50% N_2O) with a concurrent control value of 10.4 ± 2.3%. There was no significant difference between the mean number of oocytes in mice treated with 50% N_2O and in control mice. The number of abnormal sperm cells in all inhalation (air and N_2O exposed) groups was rather high. The number of abnormal sperm cells in the animals treated with saline (intraperitoneally) was 2.5 ± 0.3. N_2O exposure caused no excitement or general anaesthesia.

Viera *et al*. (1983) exposed male Wistar rats by inhalation to 0 or 0.5% N_2O/air mixtures (v/v) (0 or 9 g/m^3) for 30 days (Vie83). Immediately thereafter each male was mated with three nulliparous female rats and mated again with three more nulliparous rats after a 6-month recovery period. There was a significant reduction in mean litter size of the females (7 for the exposed group versus 12 in the control group) mated with N_2O-exposed male rats directly after the N_2O-exposure period and these offspring were smaller in size (length and body weight). Mating after a 6 months recovery period of exposed males with nulliparous females did not show these effects. General toxicity was not described in this study.

Kugel *et al*. (1990) exposed virgin female rats (Sprague Dawley rats) by inhalation for 8 h per day during 4 days (one oestrus cycle) to 30% N_2O (540 g/m^3). All N_2O-exposed rats exhibited disrupted cycles following the first day of exposure; 11 out of 12 animals went into constant proestrus for up to 3 weeks (Kug90). Control rats cycled normally. Following exposure 8 control and 8 N_2O-exposed rats were perfused, brains sectioned, and LHRH (luteinising hormone releasing hormone) cells identified by immunochemistry. A 33-fold increase in LHRH cells was noted in N_2O treated rats. In addition, 12 N_2O-exposed female rats were mated with proven male breeders. Six of the 12 N_2O-exposed rats and 12 of 12 control rats gave birth. No differences were noted in litter size or weight. General toxicity was not described in this study.

Holson *et al*. (1995) exposed adult male or female rats (Crl:COBS CD(SD) BR outbred albino) to trace concentrations of N_2O (0, 0.1, 0.5 or 1.0% in air; 0, 1.8, 9 or 18

g/m^3) for 6h/day either throughout gestation (females) of for 9 weeks (males) (Hol95). They studied effects of N_2O on male fertility by mating treated males with untreated females by examining uterine contents. There was no evidence for a substantial decline in fertility by mating exposed males. Litter size was not significantly reduced after maternal exposure, although there was a small dose-related trend for increased resorptions and decreased live births with increasing paternal N_2O exposure. Maternal and offspring weights were normal from conception through adulthood. The offspring of the treated adults was subjected to an extensive behavioural battery of tests.

Developmental toxicity

Fink *et al*. (1967) and Shepard *et al*. (1968) found defects of vertebral and rib ossification in 100% of the rat foetuses from dams (Sprague Dawley) exposed to a mixture of 45-50 % N_2O (810- 900 g/m^3), 21-25 % oxygen, and nitrogen for 2 to 6 days beginning on day 8 of gestation (Fin67, She68). A dose response relationship was observed between an increased proportion of foetal resorptions and foetal weight decrement with an increase in the number of days of exposure. Foetal length of the exposed group was significantly reduced. The sex ratio (number of males compared to the number of females) was reduced in surviving foetuses of the exposed group. Maternal toxicity was not described in this study.

Corbett *et al*. (1973) studied the effects in rats after inhalatory exposure to low concentrations of N_2O: (I) 1.5% N_2O (~27 g/m^3) for 24 h/day from gestation days 8-13, (II) 0.1% N_2O (~1.8g/m^3) for 24 h/day from gestation days 12-19, (III) 0.1% or 0.01% (~1.8 or 0.18 g/m^3) for 8 h/day from gestation day 10-13, 14-19 or 10-19 (Cob73). The number of implantations per dam was reduced and the foetal death rate increased in the dams exposed for 24 h/day to N_2O. Furthermore, the foetal death rate was increased in the dams exposed to 0.01% and 0.1% for 8 h/day during gestation days 10-13, and 0.1% group for 8 h/day for gestation days 14-19. Maternal toxicity was not described in this study.

Bussard *et al*. (1974) studied exposure to 60% N_2O (1080 g/m^3) and 0.6% halothane by inhalation for 3 h/day during gestation days 9, 10 or 11 of pregnant hamsters (Mesocricetus auratus) (Bus74). Exposure on day 11 resulted in an increased number of resorptions. Mean foetal weight and crown-rump length were decreased when females were exposed on gestation day 10 or 11.

Pope *et al*. (Pop78) studied the effects in pregnant Sprague Dawley rats during gestation (up to day 21) after exposure by inhalation to 0, 1, 10 and 50% N_2O (0, 18, 180 or 900 g/m^3) for 8 h/day. Foetal loss and skeletal and gross anomalies were not affected; in the 10 and 50% N_2O-exposed groups foetal body weight was reduced. Maternal toxicity was not described in this study.

Shah *et al*. (1979) exposed pregnant Golden Syrian hamsters during organogenesis (gestation days 7, 8, 9, 10 or 11) by inhalation (during 24 h) to different concentrations of N_2O (70-95%; 1260-1620 g/m^3) (Sha79). A significant increase in the number of resorptions was observed. Developmental effects (cleft palate, limb defects, gut herniation and foetal oedema) were observed in a small number of foetuses. A dose relationship was not observed. Maternal toxicity was not described in this study.

Lane *et al*. (1980) exposed Sprague Dawley rats for 24 h to 70-75% N_2O (1260-1350 g/m^3) on gestation day 9 and found an increased number of resorptions and malformed foetuses (gastroschisis, micro/anophthalmia, cleft lip, hydrocephaly) (Lan80). Effects were linked to vitamin B12 deficiency. Maternal toxicity was not described in this study.

Vieira *et al*. (1980) studied the effects in Wistar rats exposed to low concentrations N_2O (0. 025, 0.05, 0,1%; 0.45, 0. 9, 1.8 g/m^3) by inhalation during 24 h/day during the entire gestation period (Vie80). In the 0.1% exposure-group, a significant increase in the number of resorptions and a significant decrease in the number of live foetuses and in foetal crown-rump length was observed. Maternal toxicity was not described in this study.

Female mice (Swiss/ICR) were exposed by inhalation for 4 h/day during days 6-15 of gestation to 0, 0.5, 5 or 50% N_2O (0, 9, 90 or 900 g/m^3) (Maz 82). No adverse developmental effects were observed after treatment. Maternal toxicity was not described in this study.

Mazze *et al*. (Maz84) studied the effects of inhalatory exposure to 0, 0.75, 7.2, 25 and 75% N_2O (0, 13.5, 130, 450 or 1350 g/m^3) during 24 h on gestation day 9 in Sprague Dawley rats. After exposure to 75% N_2O, a significant increase in early and late resorptions and consistent developmental effects (e.g. runts, ocular malformations and limb deformities) were observed. During exposure to 75% N_2O, rats appeared drowsy and their motor co-ordination was impaired, food and water intake was decreased.

Koëter and Rodier (1986) studied the effects of pre-and postnatal exposure to 75% N_2O (1350 g/m^3) in DUB/ICR mice and observed retarded physical development, retarded surface and air righting (pre- and postnatal exposure) and affected total activity (postnatal exposure) (Koe86).

Tassinari *et al*. (1986) studied the effects of 75% N_2O (1350 g/m^3) by inhalation in Sprague Dawley rats after exposure during several periods of gestation (24 h/day during gestation days 11-15 or 16-20 or 8 h/day during gestation days 9-13, 11-15, 14-15 or 15 only) (Tas86). Both 24 hours exposure-periods gave a significant reduction in foetal and maternal body weight, an effect not observed after the 8 hour exposures. No gross morphological or skeletal changes were observed.

Mazze *et al*. (1986) found developmental effects (increased number of resorptions per dam, decreased foetal weight and decreased ossification) in the presence of light ma-

ternal anaesthesia after exposure of Sprague Dawley rats to 75% N_2O (1350 g/m^3) for 6 h/day by inhalation when exposed from gestation days 14-16 of gestation (Maz86).

Mazze *et al*. (1988) exposed Sprague Dawley rats on day 8 of gestation to 50-75% N_2O (900-1350 g/m^3) for 24 h alone or in combination with halothane and folate (Maz88). Exposure to N_2O alone resulted in a significantly increased number of resorptions and in major and minor skeletal abnormalities. Halothane administered in combination with N_2O protected against these effects; folinic acid (5 mg/kg body weight/day gestation days 5-13) did not.

Fujinaga *et al*. (1989) exposed Sprague Dawley rats by inhalation to 60% N_2O (1080 g/m^3) to study the susceptible period (gestation days 6, 7, 8, 9, 10, 11 or 12) for developmental effects (Fuj89). There were no differences among the groups in number of implantations and live foetuses, mean foetal weight and sex ratio. The number of resorptions was higher in the N_2O-treated groups exposed on days 8 and 11 of gestation than in the corresponding control groups. Skeletal malformations of the ribs and vertebrae were increased following exposure on day 9 of gestation. On day 8 of exposure the incidence of right-sided aortic arch and left-sided umbilical artery, abnormalities indicative of altered laterality, were increased. Female rats were mildly sedated during exposure; 15 out of 140 female rats died during the exposure period. Maternal weight was decreased among all N_2O-exposed female rats.

Rice (1990) studied behavioural effects in offspring of Swiss mice (Hla:[SW]Br) exposed by inhalation to 0, 5, 15 or 35% N_2O (0, 90, 270 or 630 g/m^3) for 4 h/day on days 6 through 15 of gestation (Ric90). Exposures did not affect reproduction indices and survival or physical milestones of development. Body weights showed significant exposure effects that could be isolated to specific exposure groups; however, N_2O-exposed mice tended to weigh more than control animals. On postnatal days (PN) 126 or 127 no effect on brain weights were observed. Ability to stay on a rotarod was not affected by prenatal N_2O exposure. Prenatal exposure to N_2O resulted in hypo-reactivity of the startle reflex on PN95 for all N_2O-exposed groups. Maternal toxicity was not described in this study.

Holson *et al*. (1995) exposed adult male or female rats (Crl:COBS CD(SD) BR outbred albino) to trace concentrations of N_2O (0, 0.1, 0.5 or 1.0% in air; 0, 1.8, 9 or 18 g/m^3) for 6 h/day either throughout gestation (females) of for 9 weeks (males) (Hol95). Litter size was not significantly reduced after maternal exposure although there was a small dose-related trend for resorptions to increase and live births to decrease with increasing paternal N_2O exposure. Maternal and offspring weights were normal from conception through adulthood. The offspring of the treated adults were subjected to an extensive behavioural battery (negative geotaxis, 23-h activity, complex maze, passive avoidance response, developmental activity, amphetamine challenge, auditory startle,

barbiturate anaesthesia). There were no significant long-term behavioural alterations in offspring exposed to trace levels of N_2O via dam or sire.

Lactation

No publications were available.

2.4 Conclusion

In human studies, some effects of N_2O were observed on fertility (Kni72, Row92, Ahl96). In other studies in man (Wyr81, And92, Kni75 and Pee 99) no effects of N_2O exposure were observed. The committee is of the opinion that the studies in man were insufficient for classification because confounding factors such as mixed gas exposure to anaesthetic, other exposures (e.g.. amalgam), differences in age between the control and the exposed groups and stress may have played a role.

In inhalatory studies in rats the following effects were observed: decreased testis weight and injury of the seminiferous tubules (20% N_2O, Kri76), reduced litter size and smaller offspring in females mated with exposed males (0.5% N_2O Vie83), disrupted cycles after exposure of females, increase in LHRH cells in the brain and decreased fertility (30% N_2O, Kug90). However, in these studies the general toxicity was not described. In other studies in mice (concentrations up to 80%) and rats (concentrations up to 80%) no effects on fertility were observed.

Therefore, based on the animal studies the committee recommends to classify N_2O in category 3 (‘substances which cause concern for human fertility’) and to label the compound with R62 (‘Possible risk of impaired fertility’).

Epidemiological studies (Ask70, Coh71, Kni72, Ros73, Ame74, Cor74a, Coh75, Pha77, Ros78, Eri79, Coh80, Lau81, Hei84, Hem85, Joh87, Row92 and Pee99) gave rise to concern about the effect of anaesthetic gas mixtures containing N_2O on abortions, foetal development, preterm birth and congenital anomalies. However, the committee is of the opinion that it is not clear whether the effects described were caused by N_2O. Confounding factors such as exposure to gas mixtures, other exposures (amalgam, radiation), differences in age between the control and the exposed groups and stress may have played a role. For that reason the quality of the studies was considered insufficient.

In several studies in rats, hamsters and mice, effects of N_2O exposure on resorptions, foetal weight and developmental anomalies were observed at concentrations between 0.01 and 95% N_2O (1.8-1620 g/m^3). In several of the studies maternal toxicity was not described. In the other studies (concentrations of up to 75% N_2O) effects on body

weight gain and mild sedation were reported. In one study (concentration N_2O 60%) maternal mortality rate of about 10% was described (Fuj89).

Therefore, in view of the animal studies with respect to the effects on development, the committee recommends to classify N_2O in category 3 ('substances which cause concern for humans owing to possible developmental toxic effects') and label the compound with R63 ('Possible risk of harm to the unborn child').

No data concerning the excretion of N_2O in human or animal milk were available.

Therefore, a lack of appropriate data precludes the assessment of N_2O for labelling for effects during lactation.

Proposed classification for fertility

Category 3, R62.

Proposed classification for developmental toxicity

Category 3, R63.

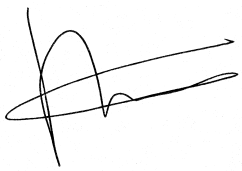
Proposed labelling for effects during lactation

Lack of appropriate data precludes assessment of N_2O for labelling for effects during lactation.

For the committee,
The Hague, 1 May 2000

dr ASAM van der Burght,
scientific secretary

dr BJ Blaauboer,
chairman



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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 and conversion factors

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 1999 for public review. No persons or organisations have commented on the draft report.

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 1.1 Fertility studies with NO_2 in man.

authors	exposure	study type/ data collection	study/ comparison 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
And92	Mixture: anaesthesia during surgery duration about 55 minutes	Cohort study; semen sampling	17 patients; preoperative sample served as control	No effect sperm volume, count motility and sperm abnormalities	Mixed exposure No covariates considered
Kni72	Mixture: waste anaesthetics Employment in anaesthesia during 1st or 2nd trimester of pregnancy	Retrospective survey UK; postal questionnaire	893 pregnancies in 563 female anaesthesists; 1,835 pregnancies in 828 female physicians	Increased infertility due to unknown causes	Mixed exposure No covariates considered
Kni75	Mixture: waste anaesthetics Employment in anaesthesia during 1st or 2nd trimester	Retrospective survey UK; postal questionnaire	Married male anaesthesists reporting at least 1 pregnancy for their wives; 5,891 pregnancies with only paternal exposure and 166 pregnancies with only maternal exposure. Male doctors registered in 1972; 7,296 pregnancies without paternal or maternal exposure	After paternal exposure: involuntary infertility negative	Mixed exposure No covariates considered
Row92	N_2O at least 5 hours scavenged or unscavenged exposure in dentistry	Retrospective survey USA 1987/1988 telephone interviews	Female dental assistants pregnant within previous 4 years; 127 exposed to scavenged N_2O and 63 exposed to unscavenged N_2O. 215 unexposed female dental assistants who were pregnant within previous 4 years.	Mean time to conception was increased in the unscavenged group	Controlled for age, race, family income, exercise, lifestyle habits and other occupational exposure.

Table 1.2 Fertility studies with NO_2 in man.

authors	exposure	study type/ data collection	study/ comparison population	investigated effects and results	remarks
Ahl96	N_2O Employment midwives who worked only day-or night time, in two-shift or three-shift rotas	Retrospective survey Sweden; postal questionnaire	972 midwives	47% became pregnant in the first cycle in the day-time group compared to 49.0%, 36.1%, and 31.9% in the permanent night, two-shift, and three-shift groups, respectively. A decreased fecundability was observed for shift work and >30 N_2O exposed deliveries per month	Controlled for reproductive history, age employment, occupational exposures, health problems, medication and lifestyle habits
Pee99	Mixture: waste anaesthetics N_2O concentration: maximum 318 mg/m^3 Employment in anaesthesia	Retrospective survey The Netherlands 1990-1997; postal questionnaire	427 pregnant females (age 22-37 years) employed in anaesthesia. 1,010 pregnant females (age 22-37 years) nurses employed in department of orthopaedics, gynaecology or surgery	No effect on time to pregnancy	Mixed exposure Controlled for age, education, menstrual cycle, life style and circumstances during work.

Table 2.1 Developmental studies in man.

authors	exposure	study type/ data collection	study/ comparison population	investigated effects and results	remarks
Ask70	Mixture: waste anaesthetics	Retrospective survey Denmark; postal questionnaire	Pregnancies conceived during employment: in 229 nurse anaesthetists, 26 female anaesthesiologists, 137 wives of male anaesthesiologists/ pregnancies conceived before employment: in 85 nurse anaesthetists, 8 female anaesthesiologists and 119 in wives of male anaesthesiologists	Spontaneous abortions positive in wives of male anaesthesiologists Premature delivery positive in wives of male anaesthesiologists Sex ratio: increased number of females in female anaesthesiologists	Mixed exposure No covariates considered
Coh71	Mixture: waste anaesthetics Any exposure in operating room (OT)	Retrospective survey USA 1. 1966-1970; personal interview 2. 1965-1970; postal questionnaire	1. 67 OT nurses/ 921 general duty nurses 2. 50 female anaesthesiologists; 81 female physicians	Spontaneous abortions positive Congenital abnormalities negative	Mixed exposure No covariates considered
Kni72	Mixture: waste anaesthetics Employment in anaesthesia during 1st or 2nd trimester of pregnancy	Retrospective survey UK; postal questionnaire	893 pregnancies in 563 female anaesthesists; 1,835 pregnancies in 828 female physicians	Spontaneous abortions, stillbirth and sex ratio negative Congenital abnormalities positive	Mixed exposure No covariates considered
Ros73	Mixture: waste anaesthetics Employment in anaesthesia or scrub nurse	Retrospective survey Finland 1965-1972; postal questionnaire	257 pregnancies in 58 anaesthesia and 124 scrub nurses; 150 pregnancies in 75 casualty department and 43 intensive care unit nurses	Spontaneous abortions positive Low birth weight and congenital abnormalities negative	Mixed exposure No covariates considered

Table 2.2 Developmental studies in man.

authors	exposure	study type/ data collection	study/ comparison 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	Spontaneous abortions positive in exposed females Congenital abnormalities positive in exposed females and wives of exposed males	Mixed exposure Maternal age and smoking
Cor74a	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	Congenital abnormalities positive	Mixed exposure Age
Coh75	Mixture: waste anaesthetics Exposure to anaesthetics at least 3 hours weekly during calendar year preceding wife's pregnancy	Retrospective survey USA; postal questionnaire	1,668 male dentists and oral surgeons exposed to anaesthetics. 1,560 male dentists and oral surgeons not exposed to anaesthetics.	Spontaneous abortion positive Congenital abnormalities negative	Mixed exposure age and smoking habit of wife at time of pregnancy
Kni75	Mixture: waste anaesthetics	Retrospective survey UK; postal questionnaire	Married male anaesthesists reporting at least 1 pregnancy for their wives; 5,891 pregnancies with only paternal exposure and 166 pregnancies with only maternal exposure. Male doctors registered in 1972; 7,296 pregnancies without paternal or maternal exposure	Spontaneous abortion positive after maternal exposure; negative after paternal exposure Congenital malformations positive after paternal exposure and negative after maternal exposure	Mixed exposure No covariates considered

Table 2.3 Developmental studies in man.

authors	exposure	study type/ data collection	study/ comparison population	investigated effects and results	remarks
Kni75	Mixture: waste anaesthetics OT employment during 1st trimester of pregnancy	Case-control study UK; postal questionnaire	1) 4,074 pregnancies with paternal exposure; 4,074 pregnancies without paternal exposure. 2a) 435 pregnancies with maternal exposure; 435 pregnancies without paternal or maternal exposure. 2b) 368 pregnancies with maternal exposure; 772 pregnancies without paternal or maternal exposure.	1) Spontaneous abortions and still birth negative; Congenital abnormalities positive 2 a and b) Spontaneous abortions positive and stillbirth negative 2a) Congenital malformations positive 2b) Congenital malformations negative	Mixed exposure Matched on maternal smoking habits, birth order, maternal and paternal age
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	Spontaneous abortion, low birth weight, and congenital abnormalities (cardiovascular) positive Stillbirth increased	Mixed exposure 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)	Spontaneous abortions negative Low birth weight positive Congenital abnormalities (musculoskeletal) increased	Mixed exposure Smoking habits
Eri79	Mixture: waste anaesthetics women 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 pregancies; 19,127 women employed in medical work who delivered in 1973 or 1975	Birth weight, perinatal death rate, congenital abnormalities negative Pregnancy duration in weeks decreased	Mixed exposure Maternal age and parity

Table 2.4 Developmental studies in man.

authors	exposure	study type/ data collection	study/ comparison population	investigated effects and results	remarks
Coh80	N_2O exposure Exposure in year preceding pregnancy; light exposure was 1- 8 hours weekly and heavy exposure was more than 8 hours weekly	Retrospective survey USA; postal questionnaire	Wives of 21,634 male dentists and 21,202 female chairside assistants/ Those not exposed in any years before conception, including year of conception	Spontaneous abortion positive in wives of male dentists and female chairside assistants also when they are exposed to N_2O only; Congenital abnormalities negative in wives of male dentists and positive in female chairside assistants also when they are exposed to N_2O only.	Exposure to inorganic mercury Maternal age and smoking history and history of previous spontaneous abortion or congenital abnormalities
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	Spontaneous abortions, sum of all abnormal pregnancies, premature birth, stillbirth and congenital abnormalities negative. Sex ratio increased number of males	Mixed exposure Maternal smoking habits
Hei84	N_2O exposure Dental assistants in poorly ventilated clinics	Historical prospective study Denmark; women entire reproductive life before 1980	352 pregnancies in dental assistants; 255 N_2O-exposed and 97 not N_2O- exposed; control group comparable with respect to work exposures and movements	Spontaneous abortions negative	Controlled for gravidity, pregnancy order and age. Amalgam exposure
Hem85	Mixture: waste anaesthetics Postal questionnaire concerning exposure during 1^{st} trimester	Case-control Finland 1973-1979; registry data for outcomes	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	1) Spontaneous abortion negative 2) Congenital abnormalities negative	1 and 2. Mixed exposure Matched on maternal age and other potential exposures

Table 2.5 Developmental studies in man.

authors	exposure	study type /data collection	study/ comparison population	investigated effects and results	remarks
Joh87	Mixture: waste anaesthetics and N_2O	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	Spontaneous abortion increased in female veterinarian assistants and positive in wives of female veterinarians exposed to N_2O and negative in female veterinarians. Spontaneous abortion increased in female veterinarians and veterinarian assistants exposed to waste anaesthetics and negative in wives of male veterinarians. Congenital abnormalities negative in female veterinarians	
Row92	N_2O at least 5 hours scavenged or unscavenged exposure in dentistry	Retrospective survey USA 1987/1988; telephone interviews	Female dental assistants pregnant within previous 4 years; 127 exposed to scavenged N_2O and 63 exposed to unscavenged N_2O; 215 unexpected female dental assistants who were pregnant within previous 4 years.	Spontaneous abortion increased in the unscavenged group	Controlled for age, race, family income, exercise, lifestyle habits and other occupational exposure.
Pee99	Mixture: waste anaesthetics N_2O concentration: maximum 318 mg/m^3 Employment in anaesthesia	Retrospective survey The Netherlands 1990-1997/postal questionnaire	427 pregnant females (age 22-37 years) employed in anaesthesia; 1,010 pregnant females (age 22-37 years) nurses employed in department of orthopaedics, gynaecology or surgery	Increased risk for abortion, preterm birth and congenital abnormalities	Mixed exposure Controlled for age, education, menstrual cycle, life style and circumstances during work.

Table 3.1 Fertility studies in animals with N_2O.

authors	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs/effects on reproduction	remarks
Kri76	LEW/f Mai rats 4-6 males/ group	1,2,3,4,5,7,10,14,21, 28,32,35 days 8 h/day or continuously for 24h	0 and 20% N_2O (inh)	Not described	Decreased testis weight after 28 days exposure in both exposure groups (8/24h). After 14 days injury to seminiferous tubules in both exposure groups. No change in plasma testosterone level	
Lan81a	(C57B1/C3H)F1 mice 5 males/group	4h/day during 5 days Sacrifice 28 days after exposure	0, 8 or 80% N_2O (inh)	Not described	No change in abnormal spermatozoa	
Maz82	Swiss /ICR mice 18-20 males/group	4h/day, 5 days/week for 9 weeks followed by a 1 week mating period with 2 untreated females/male	0, 0.5, 5 or 50% N_2O (inh)	Not described	No effects on fertility	
Maz83	Swiss Webster mice 14-15 males/ group 9 saline controls 15 females/ group	4h/day, 5 days/week for 14 weeks	0, 0.5, 5 or 50% N_2O (inh)	No excitement or general anaesthesia	No differences in testis weight, abnormal shaped sperm, sperm count or histological appearance of the testis. No significant difference between the number of oocytes of the group treated with 50% N_2O and the control group	The percentage of abnormal sperm in the saline control was much lower than in the air control 2.5 ± 0.3 versus 10.4 ± 2.3

Table 3.2 Fertility studies in animals with N_2O.

authors	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs/effects on reproduction	remarks
Vie83	Wistar rats 12 males/ group	6h/day, 5 days/week for 30 days immediately thereafter each male was mated with 3 nulliparous females. Males were mated again after a 6 months recovery period	0 or 0.5% N_2O (inh)	Not described	In the females mated immediately after exposure of the males: reduced litter size (7 versus 12 pups in the controls) and smaller offspring. No effects after recovery period.	
Kug90	Sprague Dawley rat 32 females/ group; 10 N_2O females exposed in proestrus	8h/day during 4 days	0 or 30% N_2O (inh)	Not described	All N_2O-exposed females exhibited disrupted cycles. 33-fold increase in LHRH cells in brains of N_2O-exposed females Decreased fertility	
Hol95	Crl:COBS CD(SD) BR (outbred albino) rat 10-12 males/group	paternal study 6h/day 5 days/week for 9 weeks Males were mated with 2 untreated females	0, 0.1, 0.5 or 1% N_2O (inh)	No effect on body weight	No effect on conception rate, total number of implants/litter, live foetuses/litter or resorptions/litter	

Table 4.1 Developmental toxicity studies in animals with N_2O.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Fin67, She68	Sprague Dawley rat 6 control females 3, 7, 2 females after 2, 4 or 6 days exposure, respectively	2, 4 or 6 days exposure beginning on day 8 of gestation	0 or 45-50% N_2O (inh)	Not described	Increased number of resorptions, decreased foetal weight with increase in number of days of exposure	Very small number (3 and 2) of animals after 2 and 6 days exposure
Cor73	rats (strain not described) 7-12 pregnant females/group	1. 24 h/day GD 8-13 2. 24h/day GD 12-19 3. 8h/day GD 10-13 4. 8h/day GD 14-19 5. 8h/day GD 10-19	1. 0 or 1.5% N_2O 2. 0 or 0.1% N_2O 3. 0.01 or 0.1% N_2O 4. 0.01 or 0.1% N_2O 5. 0, 0.01 or 0.1% N_2O (inh)	Not described	1. Decreased number of implantations Increased foetal death rate 2. Decreased number of implantations Increased foetal death rate 3. Increased foetal death rate 4. Increased foetal death rate	
Bus74	Hamster (Mesocritetus auratus) 8-9 pregnant females/group	3h/day during GD 9, 10 or 11	0 or 60% N_2O and 0.6% halothane (inh)	females became immobile and asleep after 15-30 min in chamber; when removed from chamber females became mobile after 5 minutes	Exposure GD 10: decreased foetal weight and crown-rump length. Exposure GD 11: Increased number of resorptions and decreased foetal weight and crown-rump length.	Mixed exposure
Pop78	Sprague Dawley rats 7-10 pregnant females/group	8h/day during the gestation period (21 days)	0, 1, 10 or 50% N_2O (inh)	Maternal toxicity not described	10 and 50% group decreased foetal weight	

Table 4.2 Developmental toxicity studies in animals with N_2O.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Sha79	Golden Syrian Hamsters 5 females/group	24h during GD 7, 8, 9, 10 or 11	0, 70, 80, 90 or 95% N_2O (inh)	Not described	Increased (NS) number of malformations no relation to dose or day of exposure GD 7 95%: Increased number of resorptions GD 10 90%: Increased number of resorptions GD 11 90%: Increased number of resorptions	
Lan80	Sprague Dawley rats 30- 31 pregnant females/group	24h on GD 9	0 and 75% N_2O (inh)	Not described	Increased number of resorptions and malformed foetuses	
Vie80	Wistar rats 12 females/group	24h during the entire gestation period	0, 0.025, 0.05 or 0.1% N_2O (inh)	Not described	0.1% group increased number of resorptions; decreased number of live foetuses and foetal crown-rump length	
Maz82	Swiss ICR mice 24-34 females/group	4h/day GD 6-15	0, 0.5, 5 or 50% N_2O (inh)	Not described	No adverse developmental effects	
Maz84	Sprague Dawley rats Groups 1, 2 and 4 time-mated females obtained from breeder, group 3 was mated in-house. About 26 females/ group	1. 24 h on GD 9 2. 24 h on GD 9 3. 24 h on GD 9 4. 24 h on GD 9	1. 0 or 75% 2. 0, 0.75, 7.5 or 75% 3. 0 and 75% 4. 0 and 25% and food deprivation on GD 9 N_2O (inh)	0.75, 7.5 and 25 % no effect 75% group: rats appeared drowsy, impaired motor coordination and decreased food and water intake	Combined data of the 4 experiments showed : 7.5-75% group increasing frequency with increasing concentration of extra lumbar rib 75%group: increased number of early and late resorptions; increased number of runts, ocular malformations, limb deformities	

Table 4.3 Developmental toxicity studies in animals with N_2O.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Koe86	DUB/ICR mice (n=5-6/group; dams or litters)	Females GD 14 6h/day Litters PN 2 exposed 4h/day Females exposed on GD 14 were allowed to litter. Physical landmarks: • ear opening • eye opening Behavioural measures: • surface righting • locomotion • air righting • general activity	0 and 75% N_2O (inh)	After exposure on GD 14 females resumed activity within a few minutes.	• no effect on pup body weight; except for an increased pup weight on PN 2 • ear unfolding retarded after pre- and postnatal 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 affected postnatal exposure	
Tas86	Sprague Dawley rats 3-7 exposed females	24 h/day GD 11-15 or 16-20 or 8h/day GD 9-13, 11-15	0 or 75% N_2O (inh)	24h GD 11-15 and 16-20: reduced maternal body weight 8h: no effect	24h GD 11-15 and 16-20: reduced foetal weight; no other developmental effects 8h	Small number of exposed females

Table 4.4 Developmental toxicity studies in animals with N_2O.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	remarks
Maz86	Sprague Dawley rats 19-25 females/ group	GD 8-10, 11-13 or 14-16	0 or 75% N_2O (inh)	Light general anaesthesia; decreased weight gain	GD 14-16: increased number resorptions and decreased foetal weight	
Maz88	Sprague Dawley rats 20-30 females/ group	24 h on GD 8	0 or 50-75% N_2O (inh)	decreased weight gain	increased number of resorptions and increased number of malformations (right-sided aortic arch) and minor skeletal anomalies (vertebral and rib anomalies)	
Fuj89	Sprague Dawley rats 20 females/group	24h on GD 6, 7, 8, 9, 10, 11 or 12	0 or 60% N_2O (inh)	Females mildly sedated 15 out of 140 females died during the exposure Maternal weight was decreased	GD 8: increase in right-sided aortic arch and left-sided umbilical artery GD 8 and 11: increased number of resorptions GD 9: skeletal malformations increased	
Ric90	Swiss (Hla:[SW]Br) mice 10 litters/group were studied	4h/day GD 6-15 Females were allowed to litter	0, 5, 15 or 35% N_2O (inh)	Not described	No reproductive effects. N_2O-exposed F1- mice tend to weight more than air exposed mice. No effect on brain weight. No effect on rotarod performance. Hyporeactivity in startle reflex on PN 95 in all N_2O-exposed animals	
Hol95	Crl:COBS CD(SD) BR outbred albino rat 10-12 males and females/group	males 6h/day 5 days/week for 9 weeks Females 6h/day during gestation Behavioural measures in F1-pups	0, 0.1, 0.5 or 1% N_2O (inh)	No effect on body weight males and females	No effect on live births after maternal exposure. Small (NS) on live births with increasing paternal exposure. No effect on long-term behavioural alterations in the offspring after maternal or paternal N_2O exposure	

Annex **E**

Abbreviations and conversion factors

Abbreviations used:

bw	body weight
d	day
F	female(s)
GD	Gestation day
i.p.	intraperitoneal
i.v.	intravenous
M	male(s)
n	number of animals
no	number
ns	not significant
NOAEL	no adverse effect level
OECD	Organisation for Economic Cooperation and Development
OT	Operating room
PN	postnatal
w	week

Conversion factors

1%	10.000 ppm
1 ppm	1.8 mg/m^3