Methanol

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 methanol. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb deze publicaties heden ter kennisname aan de minister van Volksgezondheid, Welzijn en Sport, de minister van Sociale Zaken en Werkgelegenheid en de staatssecretaris van Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

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Methanol

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 Secreatry of Social Affairs and Employment

No. 2006/04OSH, The Hague, June 13, 2006

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Samenvatting

In het voorliggende advies heeft de Gezondheidsraad methanol onder de loep genomen. Methanol wordt gebruikt bij synthese van uiteenlopende verbindingen, waaronder formaldehyde, methyl methacrylaat en methylamines. Het wordt ondermeer toegepast als oplosmiddel, verfverdunner en antivries.

Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de raad, kijkt naar effecten op de vruchtbaarheid van mannen en vrouwen zowel als op de ontwikkeling van het nageslacht. Bovendien worden effecten van blootstelling van de zuigeling via de moedermelk beoordeeld.

Op basis van Richtlijn 93/21/EEC van de Europese Unie doet de commissie een voorstel voor classificatie. Voor methanol komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om methanol niet te classificeren wegens onvoldoende geschikte gegevens.
- voor effecten op de ontwikkeling adviseert de commissie methanol te classificeren in categorie 2 *(stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken)* en met T;R61 te kenmerken.

- voor effecten tijdens de lactatie adviseert de commissie om methanol niet te classificeren wegens onvoldoende geschikte gegevens.

Executive summary

In the present report the Health Council of the Netherlands reviewed methanol. Methanol is used as feedstock in the synthesis of various compounds, amongst others formaldehyde, methyl methacrylate and methylamines. It has been used as a solvent, paints thinner and as antifreeze.

This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Committee for Compounds toxic to reproduction, a committee of the Health Council, evaluates the effects on male and female fertility and on the development of the progeny. Moreover, the committee considers the effects of a substance on the lactation and effects on the progeny via lactation.

Recommendations for classification by the committee are made in accordance with Directive 93/21/EEC of the European Union. The committee's recommendations for methanol are:

- for effects on fertility, the committee recommends not classifying methanol due to a lack of appropriate data.
- for developmental toxicity, the committee recommends classifying methanol in category 2 (*substances which should be regarded as if they cause developmental toxicity in humans*) and to label methanol with T;R61.
- the committee is of the opinion that a lack of appropriate data precludes the labelling of methanol for effects during lactation.

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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 methanol 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 APM Wolterbeek at the Toxicology and Applied Pharmacology department of TNO Quality of Life, Zeist, The Netherlands, by contract with the Dutch Health Council. 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 2005, 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 cat-

egory 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 at 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 the dosage), the labelling for effects during lactation is based on a risk characterisation and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration exceeded of the 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 Current Contents and Medline, starting from 1966 up to 2004 and by searches on internet. 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 choose to describe both human and animal data in the text. The animal data with respect to fertility and development are described in more detail in Annex D as well. Of each study, the quality of the study (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

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 (Niesink *et al.*[1]) 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.

Chapter 2

Methanol

2.1 Introduction[2,3]

Name	:	Methanol
CAS-no	:	67-56-1
Synonyms	:	carbinol, columbian spirits, hydroxymethane, methyl alcohol, methyl hydrate, methyl hydroxide, methylol, monohydroxymethane, wood alcohol, wood naphtha, and wood spirit.
Use	:	Methanol is used mainly in chemical synthesis, predominantly in the production of formaldehyde, methyl t-butyl ether, acetic acid, dimethyl terephthalate, and methyl methacrylate. It is also used as a feedstock for other organic compounds such as dimethyl ether, methylamines, methyl halides, and glycol methyl ethers, as a solvent, as antifreeze, in refrigeration systems, and as an ingredient in products such as shellacs, paints, varnishes, paints thinners, and automotive windshield washer fluids and as a denaturant for ethanol. The use of methanol in gasoline is currently limited, but increased use of alternative fuels could result in much greater use of methanol in the future. Furthermore, humans are exposed to methanol through foods (fruits, vegetables and fermented spirits) and through metabolism of food additives (aspartame).
Mol weight	:	32.04
Chem formula	:	CH_3OH
Conversion factor	:	1 ppm = 1.31 mg/m^3; 1 mg/m^3 = 0.76 ppm (at 25°C)

General toxicity	:	There is a lot of information about the effects of methanol in humans following accidental or intentional exposure. Exposure to methanol may result in nausea, dizziness, metabolic acidosis, and toxicity to the visual system (including blindness), motor disturbances, and even death. Toxicity of methanol varies greatly between species, being highest in species with a relatively poor ability to metabolise formic acid/formate (see kinetics). In cases of poor formic acid/formate metabolism, fatal methanol poisoning occurs as a result of metabolic acidosis, and neural toxicity, whereas, in animals that readily metabolise formic acid/formate, central nervous system depression (coma, respiratory failure) is usually the main toxic effect.
Exposure limit	:	In the Netherlands, the current administrative occupational exposure limit for methanol is 260 mg/m^3 (200 ppm) for a 8 h day and for a 40 h working week.
Kinetics	:	Following oral, inhalatory or dermal exposure, methanol is rapidly absorbed and metabolised primarily in the liver in a series of oxidation steps to sequentially form formaldehyde, formic acid/formate and carbon dioxide. Formic acid/formate is primarily oxidized to carbon dioxide through a tetrahydrofolate-dependent pathway. The availability of tetrahydrofolate, derived from folic acid, is the major determinant of the rate of formic acid/formate metabolism. In man and non-human primates, the folate-mediated oxidation of formic acid/formate proceeds at lower rate than the one observed in rodents, rabbits, and dogs. In rats, the oxidation of formic acid/formate exceeds the maximal rate at which methanol is converted to formic acid/formate whereas in man and non-human primates the formation of formic acid/formate exceeds its oxidation. There is a lot of evidence that formic acid is the main metabolite of methanol responsible for the visual effects and metabolic poisoning as observed in man and non-human primates. In non-human pregnant primates, the kinetics of methanol is comparable to the kinetics in non-pregnant animals. In pregnant rats and mice, there are indications that penetration of methanol to the foetal compartment decreased as the methanol exposure increased. This is possibly due to a methanol induced decrease in blood flow to the foetus.

2.2 Human studies

Fertility

No studies were found regarding the effects of exposure to methanol on human fertility.

Developmental studies

Hantson *et al.*[4] reported a case of a 26-year-old woman who ingested 250-500 ml methanol in the 38th week of pregnancy. Five hours after methanol ingestion, the woman was slightly acidotic and had a serum methanol level of 2300 mg/l and a formic acid concentration of 336 mg/l. Treatment consisted of ethanol and bicar-

bonate administration together with hemodialysis. Six days later, the woman gave birth to an infant with no signs of distress. A 10-year follow-up of the child revealed no visual disturbances.

Lorente *et al.*[5] investigated the role of occupational maternal exposure during pregnancy in relation to oral clefts. Information about the occupational exposure of 851 women (100 mothers of babies with oral clefts and 751 mothers of healthy referents) who worked during the first trimester of pregnancy was obtained from an interview. This interview was blindly reviewed by industrial hygienists, who assessed the presence of chemicals and the probability of exposure. All women were part of a multicenter European case-referent study conducted using 6 congenital malformation registers between 1989 and 1992. The odds ratio (OR) for cleft lip (with or without cleft palate) was 3.61 (95% CI 0.91-14.4). Due to the limited number of subjects, the committee is of the opinion that this result must be interpreted with caution.

Bharti[6] reported a case study of a woman who was exposed to carburetor-cleaner fluid by inhalation repeatedly during her pregnancy. On several occasions during pregnancy (16 and 27 weeks of gestation), she was admitted to the hospital because of acute intoxication (severe anion gap hyperosmolar metabolic acidosis showing blood methanol levels of about 450 mg/l). At 31 weeks of gestation, she was found obtunded and given sodium bicarbonate, to correct acidosis, and ethanol, followed by an emergency Cesarean section for acute foetal distress. At birth, the infant was of appropriate weight but presented acute foetal distress with significant metabolic acidosis. Initial hypotonia was followed by generalised hypertonicity of lower extremities within a week after birth. Neurosonogram showed bifrontal cystic lesions in the frontal area. The frontal cysts measured 1 cm x 1 cm on the right side and 0.8 cm x 0.9 cm on the left side. Magnetic resonant imaging performed on day 3 after birth showed extensive bifrontal cystic leukomalacia with some cortical atrophy and the areas of leukomalacia not communicating with the ventricles. Ventricular size was normal. There was no midline shift. The infant passed an initial hearing screen for both ears.

Lactation

No studies were found regarding the effects of exposure to methanol on human lactation.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarise the fertility and developmental studies with methanol in experimental animals.

Fertility studies

Burbacher *et al.*[7] studied the reproductive and developmental effects (see also developmental toxicity studies) of inhalatory methanol exposure in two cohorts of female *Macaca fascicularis* monkeys. The animals were exposed to methanol 0 (n=11), 200 (n=12), 600 (n=11) and 1800 (n=12) ppm (0, 262, 786, 2358 mg/m^3, respectively) for 2.5 h/day, 7 days/week during premating (about 120 days), mating (about 65 days) and gestation (about 163 days). Males were not exposed to methanol. Maternal body weights were weighed weekly and clinical observations were performed daily. Menstrual cycles were evaluated every day prior to and during exposure. Rate of conception, weight gain during pregnancy, pregnancy and delivery complications, pregnancy duration and live- and still-births were recorded. In general, females were allowed to deliver unless complications necessitate a Cesarean-section. No effect of methanol exposure was observed on female body weights, clinical observations, menstrual cycles, conception rate and live-birth index. The duration of gestation was decreased in all treatment groups (168, 160, 162 and 162 days in the 0, 200, 600 and 1800 ppm groups, respectively) but still within the normal range for this strain of animals. Some delivery complications were noted (vaginal bleeding with no signs of labor and unproductive labour for at least 3 nights) that required a Cesarean section but, most probably, these findings were not related to methanol exposure.

Effects on serum concentrations of hormones

Cameron *et al.*[8] studied the effect of inhalatory methanol exposure (0, 200, 2000, 10000 ppm (0, 262, 2620, 13100 mg/m^3) for 8 h/day, 5 day/week for 1, 2, 4 and 6 weeks) on reproductive hormones in groups of 5 male Sprague Dawley rats. Animals were sacrificed 16 h after the last exposure to determine serum levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Serum levels of testosterone were statistically significantly decreased in the low dose group at week 2 and 6 and in the mid dose group at week 6. A statistically significant increase in serum LH levels was observed in the animals of the high dose group at week 6 (LH was not measured at the other weeks). In an additional

study to determine the mechanism of decreased serum testosterone levels, male animals (5/group) were exposed to 200 ppm (262 mg/m^3) methanol for 6 weeks. Following the last exposure, the rats were given an *iv* injection with [^{14}C]testosterone. The authors concluded that methanol had no effect on the rate of testosterone removal from the blood, indicating that methanol might have a direct effect on testicular testosterone production.

In a second study of Cameron *et al.*[9], Sprague Dawley rats (5/group) were exposed by inhalation to 0 and 200 ppm methanol (0, 262 mg/m^3), 6 h/day for 1 or 7 days. Animals were sacrificed immediately or 18 h after the last exposure to measure serum levels of testosterone, LH and corticosterone. After the 1-day exposure, serum testosterone levels were statistically significantly decreased immediately after exposure and returned to control values after 18 h. No effects were observed after the 7-days exposure.

Cooper *et al.*[10] performed two studies with Long-Evans rats (n=10/group) in which the acute effects of inhalatory methanol on male sex hormones (LH, FSH, testosterone, prolactin) were determined. In the first study, the concentration of methanol was 0, 200, 5000 and 10000 ppm (0, 262, 6550, 13100 mg/m^3) for 6 h. In the second study, the concentration was 0, 5000 ppm (0, 6550 mg/m^3) for 1, 2 and 6 h. Hormone levels were determined just after exposure (study 1 and 2) and 18 h after the end of exposure (study 1). Furthermore, half of the animals were acclimated (2 weeks prior to handling) to the experimental conditions and the other half was not-acclimated. No effects on body weight and testis weights were observed. Significant effects of methanol exposure were observed on serum levels of hormones, but the direction and magnitude of the effects were strongly dependent on whether or not the animals had been acclimated to the test situation.

Effects on reproductive organs

The subchronic effects after inhalatory exposure to methanol was evaluated in rats and monkeys by Andrews *et al.*[11]. Male and female CD rats (n= 5/sex/group) and male and female cynomolgus monkeys (*Macaca fascicularis*) (n=3/sex/group) were exposed for 4 weeks to 0, 500, 2000 or 5000 ppm (0, 655, 2620, 6550 mg/m^3) of methanol for 6h/day, 5 days/week. At sacrifice, among other organs, the testes, epididymides and ovaries were weighed and macroscopically examined. In both species, no effects on body weights were observed. In rats, increased incidence of discharges around the eyes and nose were observed. In monkeys, there were no treatment-related clinical observations. Macroscopic

observations and reproductive organ weights revealed no effects of methanol among the groups.

Lee *et al.*[12] studied the effect of methanol on serum concentrations of testosterone and testis and seminal vesicle weights in male Sprague Dawley rats (n=9-10/group) exposed to 200 ppm (262 mg/m^3) of methanol 8h/day, 5 days/week for 1, 2, 4 or 6 weeks. Animals were sacrificed on the last day of exposure. No effect on body weights, macroscopic examination and weight of reproductive organs and testosterone concentrations were observed. In an additional study, the age-dependent effect of methanol on testis morphology was studied in Long-Evans rats (n=8-13 group). The younger animals were 10 months old at the end of the exposure period (0, 50, 200 and 800 ppm (0, 65.5, 262, 1048 mg/m^3) for 20 h/day for 13 weeks), the older males were 18 months old at the end of the exposure period (0 and 800 ppm (0 and 1048 mg/m^3) for 20 h/day for 13 weeks). For both ages, no effects of methanol exposure were observed on body weights, testis weights, gross testicular abnormalities and incidence of testicular lesions.

In two studies of Poon *et al.*[13,14], male and female Sprague Dawley rats (10-15/sex/group) were exposed to methanol (0, 300, 3000 ppm (0, 393, 3930 mg/m^3) in the first study and 0, 2500 ppm (0, 3275 mg/m^3) in the second study) by inhalation for 6 h/day, 5 days/week for 4 weeks. No effects of methanol on clinical signs, body weights and food consumption were observed. Histopathological examination of the reproductive organs revealed no effects of methanol.

In a long-term carcinogenicity study of Soffritti *et al.*[15], methanol was administered in drinking water at concentrations of 0, 0.5, 5.0 and 20 g/l* to groups of male and female Sprague Dawley rats for 104 weeks (n=100/sex/group). Residual liquids were removed daily and bottles were refilled daily with fresh solutions. The experiment ended after 153 weeks with the death of the last animal. Upon death, a wide range of tissues and organs were sampled for histopathological examinations, including the reproductive organs. No effects were observed in food consumption. Water consumption of female animals of the high dose group was decreased during the first 48 weeks. A slight increase in body weight of male and female rats of the high dose was observed. No substantial changes in survival or behavioural changes were observed among the groups. A dose-related increase of total malignant tumors (eg carcinomas of the ear duct, osteosarcomas of the head and hemolymphoreticular neoplasias) in the males and female groups was observed. No treatment-related non-neoplastic changes were detected by

* The dose expressed as g/kg body weight/day was calculated by the committee to be 0, 0.05, 0.5 and 2.0 g/kg body weight/day

gross inspection or histopathological examination. In the reproductive organs of the animals of the high dose group, a statistically significant increase of testicular interstitial hyperplasias, testicular adenomas and sarcomas of the uterus was observed.

As a part of a study into the effects of formalin, Ward *et al.*[16] examined the effect of methanol on sperm morphology. Crl:B6C3F1 mice were exposed by gavage to 0 (n=5) or 1.0 (n=10) g/kg body weight/day methanol for 5 days. The incidence of sperm cells showing 'banana-like-sperm-head' was statistically significantly increased (4.0 abnormalities per animal (500 sperm scored per animal). The total number of abnormal sperm morphologies was not statistically significantly increased (1.86 $^{+}/_{-}$ 0.91).

Developmental toxicity studies

Inhalatory exposure

Nelson *et al.*[17] studied the effect of methanol, administered by inhalation to pregnant Sprague-Dawley rats (n=13-15/group). The concentration of methanol was 0, 10000 and 20000 ppm (0, 13100 and 26200 mg/m^3) and 0 and 5000 ppm (0 and 6550 mg/m^3) in two separate experiments. The lower concentrations (0, 5000 and 10000 ppm) were administered on gestation days (GD) 1-19 and the highest concentration (20000 ppm) was administered on GD 7-15 for 7 h/day. In the animals of the highest dose group, a slight unsteady gait was observed only during the first days of exposure. There were no effects on body weight and food consumption. Dams were sacrificed on GD 20 and foetuses were examined for visceral and skeletal abnormalities. Foetal weights were statistically significantly decreased in the 10000 and 20000 ppm groups. The effect in the 10000 ppm group may be caused by the increased number of foetuses. No effects were observed on the incidence of resorptions. In the 20000 ppm group, a statistically significant increase in the incidence of skeletal malformations (in cranium, vertebrae and ribs) and visceral malformations (in eye, brain [exencephaly and encephaloceles] and cardiovascular and urinary system) was observed. In total 93% of litters and 54% of foetuses were affected. In the 10000 ppm group, similar effects were observed but the increase was not statistically significant whereas no adverse effects were observed in the 5000 ppm group.

Rogers *et al.*[18] performed an inhalatory developmental toxicity study with Crl:CD-1 mice (n=5-60). Pregnant animals were exposed to 1000, 2000, 5000, 7500, 10000 or 15000 ppm (0, 1310, 2620, 6550, 9825, 13100, 19650 mg/m^3)

methanol for 7 h/day on GD 6-15. Control animals were exposed to air under similar conditions and additional control groups were unhandled or food-deprived for 7 h/day. Although in each of the 7500, 10000 and 15000 ppm groups one dam died, no treatment-related overt toxic effects were observed. No effect of methanol exposure on maternal body weight (gain) was observed. On GD 17, animals were sacrificed and subsequently, foetuses were examined for external, skeletal and visceral abnormalities (skeletal and visceral examinations of the foetuses of the 7500 and 10000 ppm groups were not performed). A dose-related and statistically significant decrease in the number of live foetuses/litter was observed in the groups exposed to 7500 ppm and higher including an increased incidence of females with fully resorbed litters at 10000 ppm and higher. Foetal body weights were decreased at 10000 ppm and higher. The incidence of foetuses showing cleft palate was statistically significantly increased at 5000 ppm and above and the incidence of litters showing exencephaly was statistically significantly increased at 5000, 10000 and 15000 ppm. The lowest dose at which an effect was observed at visceral and skeletal examinations was 2000 ppm (cervical ribs).

In a pilot study, Bolon *et al.*[19] exposed Crl:CD-1 ICR BR mice (n=5-17) to methanol (0 or 10000 ppm (0 or 13100 mg/m^3)) by inhalation for 6 h/day from GD 6-15 (period of organogenesis), GD 7-9 (period of neural tube development and closure) or GD 9-11 (period of potential and abnormal neural tube reopening). Dams were sacrificed on GD 17. Maternal body weights on GD 17 were slightly, not statistically significantly, decreased. In the foetuses of dams exposed from GD 6-15, reduced foetal body weights and increased incidences of resorptions, neural tube defects, cleft palate and digit malformations were observed. In the group exposed from GD 7-9, the incidence of resorptions, neural tube defects and cleft palate, but not the incidence of digit malformations, was increased whereas the number of live foetuses was decreased. In the group exposed from GD 9-11 only cleft palate and digit malformations but no neural tube defects were observed.

To study the concentration-response relationship for neural tube defects, in a second experiment Bolon *et al.*[9] exposed mice (n=20-27) to methanol (0, 5000, 10000 and 15000 ppm (0, 6550, 13100 and 19650 mg/m^3)) from GD 7-9 (6 h/day). In the dams treated with 15000 ppm maternal body weight gain during gestation was decreased and neurological symptoms (ataxia, circling, tilted heads or depressed motor activity) were observed on the first days of exposure. The number of resorptions was increased in all groups. In the 15000 ppm group, the number of live foetuses and foetal weight were statistically significantly decreased.

Visceral examination revealed renal variations in the 5000 ppm group; neural tube defects, renal variations, cleft palate, ocular defects and limb and tail anomalies in the 10000 and 15000 ppm groups. An additional group of mice (n=22) was exposed to 15000 ppm (19650 mg/m^3) methanol from GD 9-11 (6 h/day) to confirm the results from the pilot study that neural tube defects were not induced by exposure at this period. The dams showed neurological symptoms as described above but no effect on body weight and resorptions was observed. In this group no neural tube defects and ocular defects were observed whereas the other effects were the same as described for the foetuses treated from GD 7-9.

In a third experiment described in this paper, Bolon *et al.*[19] further narrowed the window of susceptibility to neural tube defects induced by methanol by exposing mice (n=8-22) for 1 (GD 7, 8 or 9) or 2 (GD 7-8 or 8-9) days to 15000 ppm (19650 mg/m^3) for 6 h/day. No effects on maternal body weights on GD 17 were observed but all dams showed the neurological effects described above. Resorptions were increased and, consequently, the number of live foetuses was decreased on any combination of exposure days including GD 7. Neural tube defects were observed with all combinations of exposure days that contained GD 7 and 8 and were not observed following treatment on GD 9 only.

Bolon *et al.*[20] performed an additional series of studies to investigate the pathogenesis of methanol-induced cephalic malformations. Crl:CD-1 ICR BR (CD-1) mice were exposed to methanol (0 or 15000 ppm (0 or 19650 mg/m^3) for 6 h/day) from GD 7-9 (to study foetal pathology) or GD 7-8 or GD 7-9 (to study embryonic pathology). Maternal effects included neurologic distress (ataxia, circling, tilted heads or depressed motor activity) that generally recovered within 12 h after exposure and decreased body weight during the dosing period. At sacrifice on GD 17, body weights were comparable among the groups. For foetal pathology dams were sacrificed on GD 17 (n=25 for control and n=20/group for treated animals). Weight of foetuses showing neural malformations was statistically significantly decreased whereas the weight of the foetuses without malformations was not affected. Cephalic neural tube defects, especially exencephaly, were observed in about 15% of the foetuses of the methanol-treated dams, usually in association with malformed or missing cranial bones and ocular anomalies. Histopathological examinations revealed significant differences in the thickness of the frontal cortex and its constituent layers and an apparent increase in subventricular layers, both in grossly affected but also in grossly normal foetuses of dams exposed to methanol. For embryo pathology, dams treated on GD 7-8 were sacrificed on GD 8.5 and GD 9.0 and dams treated on GD 7-9 were sacrificed on GD 9.5 and 10.5 (n=3-5/time for controls and n=4-9/time for treated

animals). Growth was delayed and various stem cell populations in the neurulating embryos were affected by methanol.

Dorman *et al.*[21] examined the kinetics of methanol and formate and the occurrence of developmental toxicity (excencephaly in foetuses and open neural tube defects in embryos) in CD-1 mice. Inhalatory methanol exposure to CD-1 mice (n=12) on GD 8 (6h, 10000 ppm) induced signs of acute methanol toxicosis (central nervous system depression and ataxia) which resolved within 1 h after the end of the exposure period. The incidence of open anterior neural tubes in GD 10 embryos was statistically significantly increased.

Stanton *et al.*[22] treated pregnant Long-Evans rats (n=5-6 litters) to 0 or 15000 ppm methanol from GD 7-19 for 7 h/day to asses the effects of prenatal exposure on postnatal neurobehavioural function up to PN day 60 (mortality, body weight, motor activity, olfactory learning, thermoregulation, T-maze learning, acoustic startle response, reflex modification audiometry, pubertal landmarks, passive avoidance, visual-evoked potentials (in general 1 pup/sex/litter/test)). During the first days of exposure, maternal body weights were decreased. No treatment-related effects were observed on pup mortality (2 dead pups at birth in control group), incidence of malformed pups (two malformed pups in one litter of methanol-treated group showing anophthalmia and agenesis of optical nerve), litter size and implantation loss but on PN day 1, 21 and 35 pup weights were slightly, but statistically significantly, lower in the methanol treated animals than in the control animals. Except for a small delay in vaginal opening no effects were observed on any of the developmental parameters measured.

As part of the study into the reproductive and developmental effects following maternal inhalation exposure to methanol in nonhuman primates of Burbacher *et al.*[7] (details of this study are described previously in Fertility paragraph), developmental effects in infants of *Macaca fascicularis* monkeys prenatally exposed to methanol were investigated. Weight of the offspring was measured daily until postnatal (PN) day 147 and then weekly. Size of the offspring (length, crown-rump length, head circumference, head length and head width) was measured weekly until PN day 84 and monthly thereafter. During the first 9 months after birth, various physical and neurobehavioural function tests were performed. No effects were observed on weight and size of the infants at birth and at nine month of age, however, severe wasting, resulting in euthanasia, was observed in two female pups of the high dose group after 12 months of age. Neurobehavioural function tests did not show significant methanol-related effects on most domains of early behavioural development. No effects were observed on early reflex responses, gross motor development, spatial and con-

cept learning and memory and social behaviour. However, methanol exposure was associated with a delay in early sensorimotor development for male infants of all dose groups and with deficits in visual recognition memory for all infants of all dose groups.

In a study of Stern *et al.*[23], Long–Evans rats (n=46/group) were exposed to 4500 ppm (5895 mg/m^3) methanol by inhalation from GD 6 until PN day 21 for 6 h/day. No effect on body weights of the dams was observed. Various (neuro)-behavioural function tests were applied to assess exposure-related effects in dams and offspring: suckling test, olfactory aversion test, motor activity test in neonates (in general 1 pup/sex/litter/test) and fixed ratio running wheel test and stochastic spatial discrimination test. Only subtle behavioural changes were observed in both adults and neonates. No effect on pup body weights was observed.

Rogers and Mole[24] conducted a phase specific inhalatory study in Crl:CD-1 mice in order to determine sensitive periods of developmental toxicity of methanol. Pregnant mice (n=12-19 group) were treated to methanol (0 or 10000 ppm (0 and 13100 mg/m^3)) for 7 h/day on GD 6-7, GD 7-8, GD 8-9, GD 9-10, GD 10-11, GD 11-12 or GD 12-13 and were sacrificed on GD 17. No overt signs of maternal toxicity were observed although body weight of dams exposed from GD 7-8 was decreased as compared to their controls. The number of live foetuses per litter was decreased in dams exposed from GD 10-11 and the number of dead and resorbed foetuses was increased on GD 6-7 and GD 7-8. Foetuses were examined for cleft palate, exencephaly and skeletal abnormalities. Cleft palate occurred mainly after exposure on GD 6-7, GD 7-8 and GD 8-9. Exencephaly occurred mainly after exposure on GD 6-7 and GD 7-8. Skeletal elements showing abnormalities were exoccipital (GD 6-7), atlas vertebrae (GD 6-7), axis vertebrae (GD 6-7), cervical rib (GD 6-7), lumbar rib (GD 7-8).

As part of the same study, Rogers and Mole[24] narrowed the window of susceptibility by exposing mice (n=12-17) to 10000 ppm of methanol for 7 h/day on GD 5, 6, 7, 8 or 9. GD 7 appeared to be the most sensitive day as was observed by the highest incidence of resorptions, exencephaly, cleft palate, axis and rib effects.

Oral exposure

Infurna and Weiss[25] studied the maternal and neonatal neurobehavioural development in Long-Evans rats exposed to 2% methanol in drinking water (about 2.5 g/kg body weight/day) on either GD 15-17 or GD 17-19 (n=10/group). No

effects were observed on water consumption, maternal body weights, length of gestation and maternal behaviour (as measured by pup retrieval). No effects were observed on litter size, pup mortality, birth weight, pup weight gain during lactation and the day of eye opening. Statistically significant effects were observed on nipple attachment on PN day 1 (the mean latency time was increased but no effect was observed on the incidence of pups that successfully attached the nipple) and on homing behaviour on PN day 10 (it took about twice as long to locate nesting material from their home cages).

Pregnant Holzman rats (n=8/group) were treated by gavage with methanol (0, 1.6, 2.4 and 3.2 g/kg body weight/day) from GD 1-8 (Cummings *et al.*[26]) and sacrificed on GD 9, 11 or 20. Depending on the stage of pregnancy, maternal, embryonic and foetal parameters were assessed. Except for decreased body weights of the dams of the high dose group on day 9, no maternal effects were observed. On day 9, gravid uterus weight was statistically significantly decreased in dams of all dose groups, the weight of the implantation sites was decreased in the mid and high dose group and the number of atypical implantation sites (sites exhibiting extravasation of blood adjacent to implantation sites) was increased in the high dose group. No effect was observed on the number of implantation sites, ovarian weight and the number of corpora lutea. The results observed on gravid uterus weight and implantation sites weight are in accordance with the observed inhibition of the decidual cell response by methanol. On day 9, no effects on serum levels of progesterone, estradiol, LH and prolactin were observed. On day 11, no effect on embryonic development (yolk sac diameter, crown rump length, head length, overall development, number of somites, viability) was observed. On day 20, there were no effects on the number of foetuses/litter, foetal weight, the number of resorptions, ovary weight, gravid uterus weight, number of corpora lutea and no foetal external malformations were observed.

Rogers *et al.*[18] treated Crl:CD-1 mice twice daily with methanol (0 and in total 4 g/kg body weight) by gavage from GD 6-15. At the end of gestation, body weights of the methanol-treated mice were lower but most probably this effect was related to the increased incidence of resorptions in this group. One animal of the methanol group died. On GD 17, animals were sacrificed and examined for resorptions, external effects and foetal weights. Foetal weights were decreased, the incidence of resorptions was increased, the incidence of live foetuses was decreased and the incidence of foetuses and litters showing cleft palate or exencephaly was increased.

In a study of De-Carvalho *et al.*[27], pregnant Wistar rats (n=10-17) were treated with methanol (2.5 g/kg body weight/day) by gavage from GD 6-15. Rats

were sacrificed on GD 21. No effect on maternal body weight was observed. The incidences of resorptions and live foetuses were not affected by methanol treatment whereas foetal weight was slightly, but statistically significantly, decreased. The incidence of foetuses showing skeletal anomalies, particularly extra cervical ribs, was statistically significantly increased in foetuses of methanol-treated dams.

Sakanashi *et al.*[28] conducted a study into the effect of the maternal folate status on the developmental toxicity of methanol. CD-1 mice were fed diets containing various concentrations of folic acid during the entire study, starting 5 weeks prior to mating. On GD 6-15, mice (n=13-29) were gavaged twice daily with methanol (a total dose of 0, 4.0 and 5.0 g/kg body weight/day). On GD 18, mice were sacrificed and foetuses were examined for external (cleft palate and exencephaly) and skeletal anomalies. In the group fed a diet containing an adequate concentration of folic acid (1200 nmol/kg diet) maternal body weights were decreased by methanol, foetal weight was decreased, the number of live fetuses in the low dose group was decreased and the incidence of foetuses showing cleft palate, exencephaly and cervical malformations was increased in all methanol-treated groups. Effects were inversely related to dietary folic acid concentration.

Fu *et al.*[29] fed CD-1 mice diets containing marginal or adequate folic acid concentration from 5 weeks prior to mating until sacrifice. On GD 6-10, mice (n=21-24) were gavaged twice daily with methanol (total dose of 0 and 5 g/kg body weight/day). On GD 18, mice were sacrificed and examined for, among other parameters, cleft palate and exencephaly. In the group fed diets with an adequate concentration of folic acid, methanol had no effect on maternal body weight. In this group, foetal body weights and the incidence of live foetuses was decreased by methanol whereas the incidences of resorptions, dead foetuses and malformed foetuses were increased by methanol. Methanol increased the incidences of cleft palate and exencephaly. Effects were inversely related to dietary folic acid concentration.

In a study of Youssef *et al.*[30], four groups of pregnant Long-Evans rats were given a single dose of methanol by gavage on GD 10 at concentrations of 0 (n=13), 1.3 (n=12), 2.6 (n=11) and 5.2 (n=10) ml methanol per kg body weight (to prevent gastric irritation rats were first gavaged with an equal volume of mineral oil).

Dams were sacrificed on GD 20. Both body weight and food consumption of the animals of the high dose group was statistically significantly decreased. Furthermore, although not statistically significantly, the food consumption of the

animals of the 2.6 ml/kg body weight group was decreased by approximately 20%. There were no signs of overt toxicity and histopathological examinations of the liver, spleen, heart, lungs and kidneys of two dams per group revealed no effects. Foetuses were examined for external, skeletal and visceral abnormalities. At all dose levels, foetal body weights were statistically significantly decreased (no dose relationship was observed) and the incidence of foetuses showing anomalies and/or variations (undescended testes, exophthalmia and anophthalmia) was statistically significantly increased.

In a study of Connelly and Rogers[31], pregnant Crl:CD-1 mice (n=6-7) were gavaged with methanol (0, 4.0 and 5.0 g/kg body weight/day) on GD 7 and sacrificed on GD 18. No significant effects were observed on maternal body weight weight. Foetal weight and the incidences of live and dead foetuses were not affected but the number of resorptions was statistically significantly increased. Skeletal examinations revealed that maternal methanol exposure can alter segment patterning in the developing mouse embryo, resulting in posteriorisation of cervical vertebrae.

In vitro studies

Andrews *et al.*[32] performed an *in vitro* study in which the sensitivity of mouse and rat embryos for methanol was compared. Sprague Dawley rat embryos (GD 9) were incubated in serum containing 0, 2, 4, 8, 12 or 16 mg/ml methanol for 24 h. CD-1 mouse embryos (GD 8) were incubated for 24 h in serum containing 0, 2, 4, 6, or 8 mg/ml methanol. The doses used were similar to *in vivo* serum methanol levels after inhalation exposure. At the end of the exposure period embryos were examined for viability, dysmorphogenesis, growth and development. Developmental defects were seen in rats (8 mg/ml) and mice (≥ 2 mg/ml). Embryolethality was seen in rats at concentrations ≥ 12 mg/ml and in mice at concentrations ≥ 6 mg/ml. In mice open neural tubes were seen. In rats abnormalities in surviving embryos included open neural tube, abnormal brain and limb bud development.

Abbott *et al.*[33] examined cleft palates in cultures of CD-1 mouse embryo craniofacial tissues (GD 12). All embryos were cultured for 4 days and exposed to methanol at concentrations of 0, 6, 8, 10, 12, 15, 18 or 20 mg/ml for 6h, 12h, 1 day or 4 days. At the end of the incubation period, the cultures were examined for morphology, fusion, proliferation and growth of the palate. Incidence and completeness of palatal fusion decreased with increasing exposure. Both DNA and protein decreased with increasing exposure to methanol. ^{3}H-thymidine dem-

onstrated exposure-dependent reduction in proliferation of palatal mesenchymal cells. It was demonstrated in this study that methanol can selectively affect specific sensitive cell populations and has effects on proliferation and cell fate.

Abbott *et al.*[34] conducted an *in vitro* study to characterize methanol effects on rat and mouse embryos and determined if increased cell death occurs at sites with abnormal gross morphology. Sprague Dawley rat embryos (GD 9.5) were exposed to methanol at 0, 8, 12, or 16 mg/ml for 24 or 48 h. CD-1 mouse embryos (GD 8) were exposed to methanol at 0, 2, 4, or 8 mg/ml for 24 h. In both species reduced growth and development and increased numbers of abnormal embryos (erratic neural seam, open neural tube, rotational defects and abnormal brain development) were observed. Mice were shown to be more sensitive than rats. Cell death was observed in rat embryos after 48 h of exposure (limited after 24h) and in mouse embryos after 24 h of exposure in regions that develop into structures displaying malformations following in vivo exposure.

Brown-Woodman *et al.*[35] investigated the effect of methanol on cultured Sprague Dawley rat embryos (GD 10). Embryos were incubated for 40h in serum containing 0 or 51.3 – 411.7 μmol/ml. At the end of the exposure period embryos were examined for viability, dysmorphogenesis, growth and development. Growth and development retardations were seen at concentrations ≥ 211.7 μmol/ml (6.774 mg/ml). At 411.7 μmol/ml (13.170 mg/ml), yolk sac blood vessel development was inhibited and embryos were severely growth and developmentally retarded.

Andrews *et al.*[36] performed an *in vitro* study with Sprague Dawley rats embryos (GD 9). Embryos were cultered for 48 h in serum containing 0 – 8.75 mg/ml methanol. After the exposure period embryos were observed for signs of toxicity. A significant dose related decrease was observed in development score, somite number, crown-rump length and head length.

Huang *et al.*[37] performed an *in vitro* study with CD-1 mice embryos (GD 8). Embryos were cultured for 24 h in serum containing 0, 4, or 8 mg/ml methanol. After the exposure period embryos were evaluated for development and dysmorphogenesis. To study incorporation of methanol into embryonic macromolecules, southern blot analysis of ^{14}C-methanol labeled embryonic DNA was performed. Besides ^{35}S-methionine incorporation into protein was measured as indication of protein synthesis. Anomalies were observed at 4 mg/ml methanol (growth and development retardation) and 8 mg/ml (severe growth and development retardation and increase in incidence of erratic neural seam, open neural tube and embryo lethality). Incorporation of labelled methanol into embryonic proteins was observed.

Harris *et al.*[38] investigated whether the higher sensitivity of mice to methanol (as compared to rats) is related to species differences in methanol metabolism. CD-1 mice embryos (GD 8) and Sprague Dawley rat embryos (GD 10) were cultured for 20h and stored for enzyme assays. Protein, catalase, alcohol dehydrogenase and formaldehyde dehydrogenase were measured. Catalase activities were similar between rat and mouse. Mouse alcohol dehydrogenase and formaldehyde dehydrogenase activities in the yolk sac were significantly lower than in the rat. Besides, heart formaldehyde dehydrogenase activities were also lower in the mouse embryo. The authors concluded that the slow maturing capacity of the mouse embryo to remove formaldehyde might provide a rationale for increased sensitivity to methanol-induced embryotoxicity and teratogenicity.

Harris *et al.*[39] investigated the effect of methanol (concentrations 12 and 24 mg/ml) added to a medium in which Sprague Dawley rats embryos (GD 10) were incubated for 20h. At the end of the culture period, embryos were observed for growth and development and cysteine (precursor in gluthatione synthesis) and the antioxidant gluthatione were measured. At both concentrations (more severe at 24 mg/ml) embryo viability, neuropore closure, length and somites were decreased. Besides embryonic bloody blisters were observed. At 24 mg/ml a significant decrease was seen in gluthatione concentrations in both the embryo (38%) and the yolk sac (83%). It was shown that methanol is dysmorphogenic and that gluthatione is important in the detoxication of methanol in the developing foetus. Most likely, during the periods of gluthatione depletion, formaldehyde accounts for the teratogenic outcome.

Lactation

In a study of Aziz *et al.*[40], pups were exposed to methanol via the lactating mother throughout the lactation period from PN day 1-21. The dams (n=80) were kept on a folic-acid deficient or sufficient diet for 14-16 weeks and were exposed to methanol via the drinking water (0, 1, 2 and 4%, v/v). In the high methanol group of rats fed a folic-acid sufficient diet, body weight gain of the pups was decreased during the lactation period. Various neuro(development) toxicity parameters (spontaneous locomotor activity, conditioned avoidance response, dopaminergic and cholinergic receptors, striatal dopamine levels, expression of growth-associated protein in the hippocampal region) were affected, showing that methanol exposure during the lactation period adversely affects brain development, especially in the folic-acid deficient fed animals.

2.4 Conclusion

No studies on the effect of methanol on human fertility were available.

In a study of Burbacher *et al.*[7], *Macaca fascicularis* monkeys were exposed to methanol during premating, mating and gestation (a total of ~350 days) for 2.5 h/day. No effects were observed on menstrual cycles, conception rate and live-birth index. The duration of gestation was decreased (within normal range) and the number of delivery complications that required a Cesarean section was, non-dose related, increased. In a series of studies with male rats[8-10,12], inconsistent results on the effects of methanol on serum hormone concentrations were observed. In studies with monkeys[11] and rats[11,13-15], an effect of methanol on the histopathology of reproductive organs was examined as part of studies into the general toxicity of methanol. In general, no effects of methanol exposure were observed on weight and histopathology of the reproductive organs. In the study of Soffritti *et al.*[15], the incidence of carcinogenic lesions of the testis and uterus was increased in methanol-treated rats. However, an increased incidence of malignant tumors on other sites (eg carcinomas of the ear duct, osteosarcomas of the head and hemolymphoreticular neoplasias) was observed as well. The committee therefore considers the carcinogenic effects on the reproductive organs as nonspecific. In a study of Ward *et al.*[16], the incidence of banana-like sperm heads was slightly, but significantly, increased in mice treated with methanol. However, the committee is of the opinion that the biological significance of this finding is not clear.

In conclusion, the committee recommends not classifying methanol with respect to effects on fertility because of a lack of appropriate data.

Limited data are available concerning the effects of exposure to methanol on development in humans. Lorente *et al.*[5] found inconclusive results on the incidence oral clefts after occupational exposure to methanol during the first trimester of pregnancy. A woman intoxicated with methanol gave birth to an infant with no signs of distress six days after intoxication[4]. Another woman gave birth to an infant of appropriated weight but presenting acute foetal distress with significant metabolic acidosis[6] and neurological lesions after exposure to a mixture of solvents containing methanol (blood levels of methanol were determined). However, the committee is of the opinion that the results of these studies are inconclusive concerning the effects of methanol on developmental toxicity in man.

Pre- and postnatal developmental toxicity of methanol was studied in rats, mice and monkeys after inhalatory or oral (gavage or drinking water) exposure.[17-21,24,27-31] In general, prenatal developmental toxicity was evidenced by decreased foetal weight, decreased incidence of live fetuses and increased incidences of resorptions, dead foetuses, exencephaly, neural tube defects, cleft palate and skeletal (cranium, vertebrae, ribs, limb, tail) and visceral malformations (eye, brain, cardiovascular and urinary system).[17-21,24,27-31]

In a number of studies[17-19,27,29-31] several of these effects were observed without overt signs of maternal toxicity. At higher concentrations, more severe effects were observed in combination with maternally toxic effects (decreased body weight (gain), unsteady gait, neurological symptoms (ataxia, circling, tilted heads, depressed motor activity)).[17,19-21,24,28,30]

The prenatal developmental toxic effects observed *in vivo* were confirmed by a series of mechanistic *in vitro* developmental toxicity studies using the rat and mouse whole embryo culture assay.[32-39]

Postnatal developmental toxicity of methanol was studied in rats[22,23,25] and in *Macaca fascicularis* monkeys[7]. In the offspring of both rats and monkeys, slight effects were observed on neurobehavioural parameters.

In view of the data concerning prenatal developmental toxicity in experimental animals, the committee recommends classifying methanol in category 2 (*substances which should be regarded as if they cause developmental toxicity in humans*) and labelling methanol with T; R61 (*may cause harm to the unborn child*).

No studies concerning the excretion of methanol in human or animal milk were available. In the study of Aziz *et al.*[40], effects on pup weight gain and neurodevelopmental toxicity parameters were described in pups exposed or methanol via the lactating mother. However, the committee is of the opinion that there are insufficient data for effects on lactation. Therefore, the committee recommends not labelling methanol for effects during lactation because of a lack of appropriate data.

Proposed classification for fertility

Lack of appropriate data precludes the assessment of methanol for effects on fertility.

Proposed classification for developmental toxicity

Catergory 2; T: R61.

Proposed labelling for effects during lactation

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

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Annexes

Annex **A**

The committee

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  Toxicologist, Institute for Risk Assessment Sciences, Utrecht
- AM Bongers, *advisor*
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- PJJM Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen

- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present document was prepared by APM Wolterbeek from TNO Quality of Life in Zeist.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

Comments on the public draft

A draft of the present report was released in 2005. The following persons and organisations have commented on the draft document:

- RD Zumwalde,
  National Institute of Occupational Safety and Health (NIOSH), USA
- E González-Fernández
  Ministerio de Trabajo y Asuntos Sociales, Spain.

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 (ter-

atogenic 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 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.

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

Annex **D**

Fertility and developmental toxicity studies

Table 1 Fertility toxicity studies in animals with methanol.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproductive organs/ effects on reproduction	Remarks
Burbacher *et al.* (1999)	Macaca fascularis monkeys (n=11-12/ group in two cohorts)	Treatment 2.5 h/d, 7 d/w during premating (~120 days), mating (~65 days) and gestation (~163 days). Body weights, menstrual cycles and reproductive indices were recorded.	0, 200, 600, 1800 ppm (0, 262, 786, 2358 mg/m^3) by inhalation	No effects on body weights and clinical observations	No effects on menstrual cycles, conception rate, live-birth index. Duration of gestation was decreased but still within normal range. Complications at delivery included vaginal bleeding without labour and long-term non-productive labour. These complications were not related to methanol treatment.	Study was part of reproductive and developmental study (see developmental toxicity studies)
Cameron *et al.* (1984)	Male Sprague Dawley rats (n=5/group)	Treatment 8 h/d, 5 d/w for 1, 2, 4 and 6 weeks. Rats were sacrificed 16 h after last exposure to measure serum levels of testosterone, LH (week 6 only) and FSH.	0, 200, 2000 and 10000 ppm (0, 262, 2620 and 13100 mg/m^3) by inhalation	Not described	200 ppm: testosterone concentration decreased in week 2 and 6. 2000 ppm: testosterone concentration decreased in week 6. 10000 ppm: LH concentration increased in week 6	
Cameron *et al.* (1984)	Male Sprague Dawley rats (n=5 group)	Treatment 8 h/d, 5 d/w for 6 weeks. After last exposure rats were IV injected with [^{14}C]-testosterone	0 and 200 ppm (0, 262 mg/m^3) by inhalation	Not described	No effect of methanol on rate of [^{14}C]-testosterone clearance from blood.	

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproductive organs/ effects on reproduction	Remarks
Cameron *et al.* (1985)	Male Sprague Dawley rats (n=5/group)	Treatment 6 h/d for 1 or 7 days. Rats were sacrificed immediately or 18 h after exposure. Serum levels of testosterone, LH and corticosterone were measured	0 and 200 ppm (0, 262 mg/m^3) by inhalation	Not described	After 1 day, serum levels of testosterone were decreased immediately after exposure and returned to control levels after 18 h. No effects after 7 days exposure.	
Lee *et al.* (1991)	Male Sprague Dawley rats (n=9-10/group)	Treatment 8 h/d, 5 d/w for 1, 2, 4 or 6 weeks. Rats were sacrificed on the last day of exposure. Testis, seminal vesicles and serum testosterone levels were examined.	0 and 200 ppm (0, 262 mg/m^3) by inhalation	No effects on body weight	No effect on weight and macroscopical examination of testis and seminal vesicles. No effect on serum levels of testosterone	
Lee *et al.* (1991)	Male Long-Evans rats (n=8-13/group) Sub-Group 1: 10 months old at sacrifice Sub-Group 2: 10 months old at sacrifce	Treatment 20 h/d for 13 weeks.	Sub-group 1: 0, 50, 200 and 800 ppm (0, 65.5, 262, 1048 mg/m^3) Sub-group 2: 0 and 800 ppm (0, 1048 mg/m^3) by inhalation	No statistically significant effects on body weight	No effects on testis weight, gross abnormalities and incidence of testicular lesions	only results of animals fed folate-sufficient diet are reported.
Cooper *et al.* (1992)	Male Long-Evans rats (n=10 group) One half of the animals was acclimated to the experimental conditions before treatment, the other half was not acclimated.	Treatment Study 1: 6 h. Sacrifice just after exposure and 18 h after exposure Study 2: 1, 2, and 6 h. Sacrifice just after exposure Concentrations of LH, FSH, testosterone and prolactin were measured in serum or testis.	Study 1: 0, 200, 5000 and 10000 ppm (0, 262, 6550 and 13100 mg/m^3) by inhalation Study 2: 0 and 5000 ppm (0 and 6550 mg/m^3) by inhalation	No effect on body weight.	No effect on testis weight. Significant effects on hormone concentrations were observed but the direction and magnitude of these effects were strongly depended on whether or not the animals has been acclimated to the experimental conditions.	
Andrews *et al.* (1987)	Male and female CD rats (5/sex/group) Male and female cynomolgus monkeys (*Macaca fascularis*) (3/sex/group)	Animals were exposed 6 h/d, 5 d/w for 4 weeks. At sacrifice, among other organs, reproductive organs were weight and macroscopically examined	0, 500, 2000 and 5000 ppm (0, 655, 2620 and 6550 mg/m^3) by inhalation	Rats: no effects on body weight, increased incidence of discharge around eyes and nose Monkeys: no effect on body weight and clinical observations	No effects on weight and macroscopic observations of reproductive organs.	

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproductive organs/ effects on reproduction	Remarks
Andrews *et al.* (1987)	Male and female CD rats (5/sex/group) Male and female cynomolgus monkeys (*Macaca fascularis*) (3/sex/group)	Animals were exposed 6 h/d, 5 d/w for 4 weeks. At sacrifice, among other organs, reproductive organs were weight and macroscopically examined	0, 500, 2000 and 5000 ppm (0, 655, 2620 and 6550 mg/m^3) by inhalation	Rats: no effects on body weight, increased incidence of discharge around eyes and nose Monkeys: no effect on body weight and clinical observations	No effects on weight and macroscopic observations of reproductive organs.	
Poon *et al.* (1994) Poon *et al.* (1995)	Male and female Sprague Dawley rats (n=10-15 group)	Rats were exposed 6 h/d, 5 d/w for 4 weeks. At sacrifice, among others, reproductive organs were sampled for histopathological examination	Poon (1994): 0, 300 and 3000 ppm (0, 393, 3930 mg/m^3) Poon (1995) 0, 2500 ppm (0, 3275 mg/m^3)	No effects on clinical observations, body weight and food consumption	No histopathological effects on reproductive organs.	

Table 2 Developmental inhalation toxicity studies in animals with methanol.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity	Remarks
Nelson *et al.* (1985)	Pregnant Sprague Dawley rats (n=15/ group)	Control, low and mid dose group were treated from GD 1-19, 7 h/d. Rats from high-dose group were treated from GD 7-15, 7 h/d. Rats were sacrificed on GD 20 and foetuses were examined for visceral and skeletal abnormalities	0, 5000, 10000 and 15000 ppm (0, 6550, 13100 and 26200 mg/m^3) by inhalation	No effects on body weights and food consumption. Animals of high dose group showed unsteady gait during first days of exposure.	10000 and 20000 ppm: decreased foetal weights 20000 ppm: 93% of litters affected; 54% of foetuses showing skeletal- (in cranium, vertebrae and ribs) and visceral malformations (in eye, brain [exencephaly and encephaloceles], cardiovascular and urninary system) 10000 ppm: similar effects as in 20000 ppm group but incidence was lower.	
Rogers *et al.* (1993)	Pregnant Crl:CD-1 mice (n=5-60)	Mice were treated from GD 6-15, 7 h/d. Additional control groups were left unhandled or food-deprived for 7 h/d. Rats were sacrificed on GD 17 and examined for external, skeletal and visceral abnormalities.	0, 1000, 2000, 5000, 7500, 10000 and 15000 ppm (0, 1310, 2620, 6550, 9825, 13100 and 19650 mg/m^3) by inhalation	One dead animal in each of 7500, 10000 and 15000 ppm group. No treatment-related effects on clinical observations and body weight.	2000 ppm: increased incidence of cervical ribs 7500 ppm: decreased number of live foetuses/litter 10000 ppm: increased incidence of fully resorbed litters; decreased foetal weights. 5000 ppm: increased incidence of cleft palate and exencephaly	No skeletal and visceral examination of foetuses of 7500 and 10000 ppm group.
Bolon *et al.* (1993)	Pregnant Crl:CD-1 ICR BR mice (n=5-17)	Mice were treated from GD 6-15 (organogenesis); GD 7-9 (neural tube development and closure); GD 9-11 (potential and abnormal neural tube reopening) for 6 h/d. Dams were sacrificed on GD 17 for external, visceral and skeletal examination	0 and 10000 ppm (0, 13100 mg/m^3) by inhalation	GD 17 maternal body weight were slightly, not statistically significant, decreased.	GD 6-15: reduced foetal weight, increased incidence of resorptions, neural tube defects, cleft palate and digit malformations. GD 7-9: Increased incidence of resorptions, neural tube defects, cleft palate. Decreased incidence of live foetuses. GD 9-11: Increased incidence of cleft palate and digit malformations.	
Bolon *et al.* (1993)	Pregnant Crl:CD-1 ICR BR mice (n=20-27)	Mice were treated from GD 7-9 for 6 h/d and sacrificed on GD 17 for foetal examinations.	0, 5000, 10000 and 15000 ppm (0, 6550, 13100, 19650 mg/m^3) by inhalation	15000 ppm: decreased body weight on GD 17 Neurological symptoms (ataxia, circling, tilted heads, depressed motor activity)	5000 ppm: increased incidence of resorptions 5000 ppm: renal variations 10000 and 15000 ppm: neural tube defects, renal variations, cleft palate, ocular defects, limb and tail anomalies.	Effect on maternal body weight most probably due to increased incidence of resorptions.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity	Remarks
Bolon *et al.* (1993)	Pregnant Crl:CD-1 ICR BR mice (n=17-22)	Mice were treated from GD 9-11 (6 h/d) and sacrificed on GD 17 for foetal examinations.	0 and 15000 ppm (0, 19650 mg/m^3) by inhalation	Neurological symptoms (ataxia, circling, tilted heads, depressed motor activity)	renal variations, cleft palate, limb and tail anomalies	
Bolon *et al.* (1993)	Pregnant Crl:CD-1 ICR BR mice (n=8-22/ group)	Mice were treated for 1 day (GD 7, 8 or 9) or 2 days (GD 7-8 or 8-9), 6 h/d, and sacrificed on GD 17 for foetal examination of neural tube defects	0 and 15000 ppm (0, 19650 mg/m^3) by inhalation	Neurological symptoms (ataxia, circling, tilted heads, depressed motor activity)	GD 7 and GD 7-8: Increased incidence of resorptions. Decreased incidence of live foetuses. GD 7, 8 and GD 7-8 and GD 8-9: Neural tube defects	Effects strongly depend on day of exposure (GD 7 and/or 8).
Bolon *et al.* (1994)	Pregnant Crl:CD-1 ICR BR mice (n=20-25/ group for foetal effects and n=3-9/ group for embryonic effects)	Mice were treated from GD 7-9, 6 h/d and sacrificed on GD 17 to study foetal effects or treated on GD 7-8 (sacrificed on GD 8.5 or 9.0) or GD 7-9 (sacrificed on GD 9.5 or 10.5) to study embryonic effects	0 and 15000 ppm (0, 19650 mg/m^3) by inhalation	Neurological symptoms (ataxia, circling, tilted heads, depressed motor activity), decreased body weight during dosing.	Foetal effects: Decreased weight of foetuses showing neural malformations. Increased incidence of neural tube defects (exencephaly), malformed or missing cranial bones and ocular abnormalities. Histopathological effects in grossly malformed but also in grossly normal foetuses. Embryonal effects: Delayed growth and stem cell populations affected.	
Dorman *et al.* (1995)	CD-1 mice (n=12/ group)	Mice were treated on GD 8 for 6 h/d and sacrificed on GD 10 for embryonic examination	0 and 10000 ppm (0 and 13100 mg/m^3) by inhalation	Central nervous system depression and ataxia	Incidence of open anterior neural tubes was increased	
Bolon *et al.* (1993)	Pregnant Crl:CD-1 ICR BR mice (n=8-22/ group)	Mice were treated for 1 day (GD 7, 8 or 9) or 2 days (GD 7-8 or 8-9), 6 h/d, and sacrificed on GD 17 for foetal examination of neural tube defects	0 and 15000 ppm (0, 19650 mg/m^3) by inhalation	Neurological symptoms (ataxia, circling, tilted heads, depressed motor activity)	GD 7 and GD 7-8: Increased incidence of resorptions. Decreased incidence of live foetuses. GD 7, 8 and GD 7-8 and GD 8-9: Neural tube defects	Effects strongly depend on day of exposure (GD 7 and/or 8).
Bolon *et al.* (1994)	Pregnant Crl:CD-1 ICR BR mice (n=20-25/ group for foetal effects and n=3-9/ group for embryonic effects)	Mice were treated from GD 7-9, 6 h/d and sacrificed on GD 17 to study foetal effects or treated on GD 7-8 (sacrificed on GD 8.5 or 9.0) or GD 7-9 (sacrificed on GD 9.5 or 10.5) to study embryonic effects	0 and 15000 ppm (0, 19650 mg/m^3) by inhalation	Neurological symptoms (ataxia, circling, tilted heads, depressed motor activity), decreased body weight during dosing.	Foetal effects: Decreased weight of foetuses showing neural malformations. Increased incidence of neural tube defects (exencephaly), malformed or missing cranial bones and ocular abnormalities. Histopathological effects in grossly malformed but also in grossly normal foetuses. Embryonal effects: Delayed growth and stem cell populations affected.	

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity	Remarks
Dorman *et al.* (1995)	CD-1 mice (n=12/ group)	Mice were treated on GD 8 for 6 h/d and sacrificed on GD 10 for embryonic examination	0 and 10000 ppm (0 and 13100 mg/m^3) by inhalation	Central nervous system depression and ataxia	Incidence of open anterior neural tubes was increased	
Stanton *et al.* (1995)	Pregnant Long-Evans rats (n=5-6 litters/ group)	Rats were treated for GD 7-19 (7 h/d) and allowed to litter. Neurobehavioural function of the pups was tested up to PN day 60 (in general 1 pup/ sex/litter/test)	0 and 15000 ppm (0, 19650 mg/m^3) by inhalation	Body weights decreased during first days of exposure.	No effects on pup mortality, malformations, litter size, implantation loss, neurobehavioural function Pup weights decreased on PN day 1, 21, 35. Delay in vaginal opening.	Number of litters studied is considered very limited for this kind of studies
Burbacher *et al.* (1999)	Macaca fascularis monkeys (n=11-12/ group in two cohorts)	Treatment 2.5 h/d, 7 d/w during premating (~120 days), mating (~65 days) and gestation (~163 days). Neurobehavioural functions tests were performed up to 9 months	0, 200, 600, 1800 ppm (0, 262, 786, 2358 mg/m^3) by inhalation	No effects on body weights and clinical observations	No effect on weight and size of the neonates. In general no significant effects on early behavioural development. Males of all groups showed delay in early sensorimotor development, male and female of all groups showed deficits in visual recognition memory.	Study was part of reproductive and developmental study (see fertility toxicity studies)
Stern *et al.* (1997)	Long-Evans rats (n=46/ group)	Rats were treated from GD 6 – PN day 21 (6 h/d) for neurobehavioural (development) testing of dams and offspring (in general 1 pup/sex/litter/ test)	0 and 4500 ppm (0 and 5895 mg/m^3)	No effect on body weight. Only subtle neurobehavioural effects.	No effect on pup body weights. Only subtle neurobehavioural developmental effects.	
Rogers *et al.* (1993)	Crl:CD-1 mice (n=12-19/ group)	Mice were treated from GD 6-7, GD 7-8. GD 8-9,GD 9-10, GD 10-11, GD 11-12, GD 12-13 (7 h/d) and sacrificed on GD 17 for foetal examination of cleft palate, exencephaly and skeletal abnormalities	0 and 10000 ppm (o and 13100 mg/m^3)	No overt signs of toxicity. Body weights of dams treated on GD 7-8 was decreased.	GD 10-11: Decreased incidence of live foetuses. GD 6-7, 7-8: Incidence of dead and resorbed foetuses increased. Increased incidence of cleft palate on GD 6-7, GD 7-8, 8-9 Increased incidence exencephaly on GD 6-7, 7-8 Increased incidence of skeletal abnormalities on GD 6-7, 7-8	
Rogers *et al.* (1993)	Crl:CD-1 mice (n=12-17/ group)	Mice were treated from GD 5, 6, 7, 8 or 9 (7 h/d) and sacrificed on GD 17 for foetal examination of cleft palate, exencephaly and skeletal abnormalities	0 and 10000 ppm (0 and 13100 mg/m^3)	No overt signs of toxicity	Exposure on GD 7 appeared to be the most sensitive days as was reflected by the highest foetal incidence of resorptions, exencephaly, cleft palate and skeletal abnormalities.	
Infurna *et al.* (1986)	Long-Evans rats (n=10/ group)	Rats were treated on either GD 15-17 or 17-19 for (neuro)behavioural (development) testing of dams and offspring	0 and 2% in drinking water (0 and ~2.5 g/ kg body weight/day)	No effect on water consumption and body weight	No effect on length of gestation, litter size, pup mortality, birth weight, pup weight gain and day of eye opening. No effect on maternal behaviour (pup retrieval). In pups significant effect on nipple attachment time and homing behaviour	

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Developmental toxicity	Remarks
Cummings *et al.* (1993)	Holzman rats (n=8/ group)	Rats were treated from GD 1-8 and sacrificed on GD 9, 11 or 20 for embryonic or foetal examination	0, 1.6, 2.4, 3.2 g/kg body weight/day by gavage	Decreased body weight of dams of high dose group	*GD 9*: No effect on number of implantations and corpora lute and ovarian weight, sex hormone levels Effects on gravid uterus weight, implantation sites weight, number of atypical implantation sites, decidual cell response. *Day 11*: No effect on embryonic development. *Day 20*: No effect on reproductive parameters and external malformations.	
Rogers *et al.* (1993)	Crl:CD-1 mice (n=4-8/ group)	Mice were treated from GD 6-15 and sacrificed on GD 17 for foetal examination	0 and 4 g/kg body weight/ day by gavage	One dead animal. Final body weight were decreased.	Foetal weights decreased. Incidence of resorptions increased, incidence of live foetuses decreased, incidence of cleft palate and exencephaly increased.	Effect on final body weight most probably due to increased incidence of resorptions
De-Carvalho *et al.* (1994)	Wistar rats (n=10-17/ group)	Rats were treated from GD 6-15 and sacrificed on GD 21 for foetal examination	0 and 2.5 g/kg body weight/ day by gavage	No effect on body weight	No effect on resorptions and incidence of live foetuses. Foetal weight decreased. Incidence of skeletal anomalies increased.	Only results of well-nourished animals are included in this report
Sakanashi *et al.* (1996)	CD-1 mice (n=13-29)	Mice were treated from GD 6-15 and sacrificed on GD 18 for foetal examination	0, 4.0 and 5.0 g/kg body weight/day by gavage	Body weights were slightly decreased.	Incidence of resorptions increased, number of live foetuses in low-dose decreased, foetal weight decreased, incidence of foetuses with cleft palate, exencephaly and cervical malformations increased	Only results of animals fed an adequate dietary concentration of folic acid are included in this report
Fu *et al.* (1996)	CD-1 mice (n=21-24)	Mice were treated from GD 6-10 and sacrificed on GD 18 for foetal examination	0 and 5.0 g/kg body weight/ day by gavage	No effect on body weight	Foetal weight and incidence of live foetuses decreased, incidence of resorptions, dead fetuses, malformed fetuses, cleft palate and exencephaly increased.	Only results of animals fed an adequate dietary concentration of folic acid are included in this report
Youssef *et al.* (1997)	Long-Evans rats (n=10-13)	Rats were treated on GD 10 and sacrificed on GD 20 for foetal examination.	Single dose of 0, 1.3, 2.6 and 5.2 ml/kg body weight by gavage.	Body weight and food consumption decreased in high dose group. No signs of overt toxicity. No effects on histopathology	All dose levels: Foetal body weights decreased, incidence of fetuses showing anomalies and/or variations (undescended testis, exophthalmia, anophthlamia) increased.	To prevent gastric irritation rats were first gavaged with an egual volume of mineral oil

Annex **E**

Abbreviations

Abbreviations used:

bw	=	body weight
d	=	day
FSH	=	Follicle stimulating hormone
GD	=	gestation day
h	=	Hours
IV	=	Intravenous
LH	=	Luteinizing hormone
n	=	number
NOAEL	=	No Observed Adverse Effect Level
OECD	=	Organisation for Economic Cooperation and Development
PN	=	postnatal
ppm	=	part per million