Health Council of the Netherlands

Ifosfamide

Evaluation of the effects on reproduction, recommendation for classification

2014/08

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Ifosfamide*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-8079/HS/cn/543-K14
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Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van ifosfamide op de vruchtbaarheid en het nageslacht; het betreft ook effecten op de lactatie en via de moedermelk op de zuigeling.

Dit advies maakt deel uit van een uitgebreide reeks waarin voor de voortplanting giftige stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste commissie van de Gezondheidsraad, de Subcommissie Classificatie reproductietoxische stoffen. Het is vervolgens getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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Ifosfamide

Evaluation of the effects on reproduction, recommendation for classification

Subcommittee on the Classification of Reproduction Toxic Substances,
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2014/08, The Hague, April 3, 2014

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).

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

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad ifosfamide onder de loep genomen. Ifosfamide wordt veel gebruikt bij chemotherapie voor veel verschillende type tumoren, zoals weke-delensarcomen, ovariumcarcinomen, osteosarcomen en haematopoietische maligniteiten. 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 Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de raad, hierna aangeduid als de commissie, kijkt zowel naar effecten op de vruchtbaarheid van mannen en vrouwen als naar effecten op de ontwikkeling van het nageslacht. Bovendien worden effecten van blootstelling van de zuigeling via de moedermelk beoordeeld.

Op basis van Verordening (EG) 1272/2008 van de Europese Unie doet de commissie een voorstel voor classificatie. Voor ifosfamide komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie ifosfamide in categorie 1B (stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting) te classificeren en met H360F (kan de vruchtbaarheid schaden) te kenmerken

- voor effecten op de ontwikkeling adviseert de commissie ifosfamide in categorie 1B (stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting) te classificeren en met H360D (kan het ongeboren kind schaden) te kenmerken
- voor effecten op en tijdens de lactatie adviseert de commissie om ifosfamide niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report, the Health Council of the Netherlands reviewed ifosfamide. Ifosfamide is widely used in chemotherapy for many different tumour types, such as soft tissue sarcomas, osteosarcomas, ovarian carcinomas and haematopoietic malignancies. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at the request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council, hereafter called the Committee, 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 lactation and on the progeny via lactation.

The Committee recommends classification according to Regulation (EC) 1272/2008 of the European Union. For ifosfamide, these recommendations are:

- for effects on fertility, the Committee recommends classifying ifosfamide in category 1B (*presumed human reproductive toxicant*), and labelling with H360F (*may damage fertility*)
- for effects on development, the Committee recommends classifying ifosfamide in category 1B (*presumed human reproductive toxicant*) and labelling with H360D (*may damage the unborn child*)

- for effects on or via lactation, the Committee recommends not labelling ifosfamide due to a lack of appropriate data.

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. This classification is performed by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The classification is performed according to European Union Regulation (EC) 1272/ 2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP guideline is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The Subcommittee'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 reproductive toxicant (category 1A and 1B and 2) or compound with effects on or via lactation.

1.2 Committee and procedure

This document contains the classification of ifosfamide by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter

called the Committee. The members of the Committee are listed in Annex A. The submission letter (in English) to the Minister can be found in Annex B.

In 2013, 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 C. The Committee has taken these comments into account in deciding on the final version of the report. The received comments, and the replies by the Committee, can be found on the website of the Health Council.

The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development as well as lactation of the above mentioned compound.

Classification for reproduction (fertility (F) and development (D)):	
Category 1	Known or presumed human reproductive toxicant (H360(F/D))
Category 1A	Known human reproductive toxicant
Category 1B	Presumed human reproductive toxicant
Category 2	Suspected human reproductive toxicant (H361(f/d))
No classification for effects on fertility or development	
Classification for lactation:	
	Effects on or via lactation (H362)
	No labelling for lactation

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC) 1272/2008) presented in Annex D. The classification of compounds 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 guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations (see Annex E).

1.3 Labelling for lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The guideline defines that substances which are absorbed by women and have been shown to 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, shall be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labelling for effects on or via lactation is based on a risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects on or via lactation when it is likely that the substance would be present in breast milk at potentially toxic levels. The Committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceeding the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.4 Data

Literature searches were conducted in the on-line databases XTOXLINE, MEDLINE and CAPLUS, up to and including January 2012 without a starting date; an update was performed in TOXNET in June 2013. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and most recent reviews were consulted. References are divided into literature cited and literature consulted but not cited.

The Committee describes both human and animal studies in the text. The animal data are described in more detail in Annex F as well. Of each study, the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

In the assessment of the potential reproduction toxic effects of ifosfamide, the Committee also used data on adverse effects related to its application as a therapeutic agent.

1.5 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 precludes assessment of the compound for reproductive toxicity
- sufficient data show that no classification for reproductive toxicity is indicated.

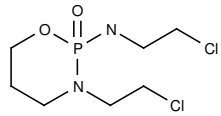
1.6 Final remark

The classification of compounds is based on hazard evaluation only (Niesink et al., 1995)[16], which is one of a series of elements guiding the risk evaluation process. The Committee emphasizes that for derivation of health-based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organizations.

Chapter 2

Ifosfamide

2.1 Introduction

chemical name	:	ifosfamide
IUPAC name	:	3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide
CAS name	:	2H-1,3,2-oxazaphosphorin-2-amine, N,3-bis(2-chloroethyl)tetrahydro-, 2-oxide
CAS registry number	:	3778-73-2
EC/EINECS number	:	223-237-3
synonyms	:	3-(2-chloroethyl)-2-[(2-chloroethyl)amino]perhydro-2*H*-1,3,2-oxazaphosphorine 2-oxide; *N*,3-bis(2-chloroethyl)-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide; iphosphamide; isophosphamide; isofosfamide; isoendoxan; isofosfamidum
colour and physical state	:	white crystals
molecular weight	:	261.1
molecular formula	:	$C_7H_{15}C_{12}N_2O_2P$
structure	:	
		phosphor is a stereocentre; 2 enantiomers (R/S)
melting point	:	48-50 °C; 39-41 °C from anhydrous ether; 72 °C
vapour pressure	:	3.96×10^{-3} Pa at 25°C (estimated)
Log P (octanol-water)	:	0.86 (experimental)

solubility	:	soluble in water (1 in 10) and carbon disulphide (1.5 in 100); very soluble in dichloromethane
use	:	in cancer chemotherapy and always administered as a racemic mixture. In the Netherlands, it can be prescribed for the treatment of small-cell and non-small-cell lung carcinomas, testicular tumours (in case of recurrence after conventional therapy), ovarian carcinomas, soft tissue sarcomas (especially leyomyo-, rhabdomyo-, chondrosarcomas) and osteosarcomas, and as second-line therapy for Hodgkin's disease and non-Hodgkin's lymphomas with an intermediate or high degree of malignancy.[3]
kinetics	:	Oral bioavailability is close to 100%. No information on bioavailability after dermal or inhalation exposure is available. Following intravenous administration, the volume of distribution of ifosfamide approximates the total body water volume, suggesting that distribution takes place with minimal tissue binding. Ifosfamide can cross the blood-brain barrier. Ifosfamide is a prodrug that needs activation via CYP3A4, a cytochrome P450 isoenzyme, to 4-hydroxy-ifosfamide. Further oxidation results in formation of ifosforamide mustard with concurrent formation of acrolein. The metabolite ifosforamide mustard can alkylate DNA and is believed to be the major cause of the cytotoxicity observed. Dechloroethylation of ifosfamide with concurrent formation of chloroacetaldehyde is also a major metabolic route accounting for 25-60% of the metabolism of ifosfamide in humans.[8]
regulations and advisories	:	In 2008, the Health Council of the Netherlands was of the opinion that ifosfamide should be considered as carcinogenic to humans, a recommendation comparable to the EU classification as a category 2 carcinogen (according to Guideline 93/21/EC of the European Union).[6] A harmonised EU GSH classification for carcinogenicity is not available. IARC concluded that there was limited evidence for the carcinogenicity of ifosfamide in experimental animals. No conclusion was drawn for the carcinogenicity in humans in the absence of data. Therefore, according to the IARC guidelines, ifosfamide was considered to be not classifiable as to its carcinogenicity to humans (Group 3).[7]

Data from[1,7,20] unless otherwise noted.

2.2 Human studies

Numerous studies are available in which ifosfamide was administered as part of multidrug chemotherapy. However, in most of the studies, the effect of ifosfamide on fertility or on developmental toxicity can or has not been evaluated. The few studies that give an evaluation of reproductive effects of ifosfamide mainly focus on male fertility.

Fertility studies

Longhi et al. investigated fertility in 96 male patients by extracting information on individual drugs by comparing different combinations of drugs. In a retrospective study in male patients treated for osteosarcoma at the Rizzoli Orthopaedic Institute in Bologna (Italy) from 1976 to 1996, the persons alive in 2001 could be divided into six groups based on different chemotherapy protocols. The median age at time of chemotherapy was 17 years (range: 10-42 years) and the median follow-up was nine years (range: 4-17 years) after the end of chemotherapy. Four drugs were administered (methotrexate, cisplatin, doxorubicin, ifosfamide) at different doses. Forty patients received combinations of methotrexate, cisplatin and doxorubicin, but no ifosfamide, and 56 patients combinations of methotrexate, cisplatin, doxorubicin and ifosfamide (44 patients ifosfamide doses of 24 to 42 g/m^2 (≈690-1,200 mg/kg bw*; 12 doses of 60 to 72 g/m^2 (≈1,710-2,060 mg/kg bw)). Twenty-six of the 96 patients (age: 21-46 years) had semen analysis after the interview (no semen analysis performed before chemotherapy). Following combination therapy including ifosfamide (median: 42 g/m^2, range: 24-72 g/m^2; ≈1200 mg/kg bw, range: ≈690-2,060 mg/kg bw), an increase in the incidence of azoospermia was found compared to treatment with combinations without ifosfamide (14/16 vs. 5/10; p=0.005). It should be noted that no tests were performed to exclude other causes of sterility. Of the other drugs used, none demonstrated a statistically significant association between dosage and the occurrence of azoospermia. Of the six patients who were normospermic, five had received no ifosfamide and one ifosfamide doses of 24 g/m^2 (≈690 mg/kg bw). Impaired libido or sexual dysfunction was not reported by any of the patients. For testosterone, LH and FSH values measured in five patients and fathering of children by seven patients, results could not be linked to ifosfamide specifically.[9]

Garolla et al. compared the effects of ifosfamide and cyclophosphamide treatment on semen characteristics in 33 males who had received chemotherapy for childhood cancer at the Oncoematology Paediatric Clinic in Padova (Italy) between 1980 and 2000. All patients had received chemotherapy at pre- or postpubertal age for soft tissues or bone sarcomas by several multidrug protocols, including cyclophosphamide (eight subjects) or ifosfamide (25

* All dose conversions in this study are rough estimations as an unknown number of children were present in each group; all conversions are based on a bw of 70 kg and a body surface of 2 m^2 for an adult.[17]

subjects). Ten patients of the ifosfamide group had received a higher dose than 60 g/m^2 (≈2,110 mg/kg bw*) and 15 a lower dose. Slow (24 hours) and rapid infusion (1-3 hours) protocols were divided equally between the low- and high-dose group. The time between end of treatment and evaluation is not exactly clear: at least two years is stated in the title of a table and at least seven years is stated in the text. Results for the ifosfamide group consisted of normal values for seminal volume, total sperm count and sperm concentration, a normal percentage of sperm aneuploidies, and normal plasma concentrations of FSH, LH, testosterone, inhibin B (marker of spermatogenesis), prolactin and oestradiol, when evaluated according to 1999 World Health Organization guidelines. When the ifosfamide group was divided based on pubertal stage at time of treatment (13 prepubertal vs. 12 postpubertal) or based on protocol used (13 slow vs. 12 rapid infusion), no differences in terms of semen concentrations, testicular size, inhibin B, LH and testosterone plasma levels were seen either. The FSH plasma concentration was increased in the rapid infusion group compared to the slow infusion group ($p<0.05$). Evaluation of sperm chromosomes by FISH (fluorescence *in situ* hybridization) analysis showed a slightly higher mean percentage of sperm aneuploidies compared to normozoospermic controls (1.8±0.7% vs. 1.6%; not statistically significant).[5]

Ridola et al. investigated gonadal function in adult male survivors who had received ifosfamide treatment in a multidrug protocol during childhood. Male patients were eligible for the study if they had received ifosfamide as the only alkylating agent. The median age at treatment was 12 years (range: 0.5-20.7 years) and the median age at investigation was 22.5 years with a median time of 8.5 years after treatment. The median cumulative dose of ifosfamide was 54 g/m^2 (range: 18-114 g/m^2; ≈1,720 mg/kg bw**, range: 570-3,640 mg/kg bw[a]). Elevated FSH values were seen in 5/60 patients having received ifosfamide at a cumulative dose of ≥48 g/m^2 (≈1,530 mg/kg bw[a]); no association with ifosfamide dose was obvious. All but two males had normal testosterone levels. LH was elevated in 14/100 patients. At the time of hormone measurement, 6/100 patients had fathered at least one child.[19]

Williams et al. evaluated gonadal function of patients enrolled in multidrug protocols for treatment of Ewing's sarcoma and soft tissue sarcoma containing

* Dose conversion in this study based on a bw of 42 kg and body surface of 1.34 m^2 for a 12-year-old boy (median age).[15]

** All dose conversions in this study based on a bw of 42 kg and body surface of 1.34 m^2 for a 12-year-old boy (median age).[15]

ifosfamide as the only potential gonadotoxic agent in twenty-two Children's Cancer Study Group Centres in the UK in the 1980-1990s. All patients were event-free survivors for more than two years, were post-pubertal and more than 15 years of age at the time of study. Boys were treated at a median age of 11.8 years (range: 5.4-21.3 years) and girls at a median age of 12.1 years (range: 3.6-15.6 years). Pubertal development, menstrual history in the girls and semen analysis in the boys were investigated after a median follow-up of ten years. All 32 boys progressed normally through puberty. No gonadal dysfunction was seen at a total ifosfamide dose of <60 g/m^2 (≈1,910 mg/kg bw*). In those with a dose >60 g/m^2 (≈1,910 mg/kg bw), 8/11 who underwent semen analysis were subfertile, 8/26 had elevated FSH levels and 13/26 showed decreased inhibin B, supporting evidence of germ cell failure. All 13 girls progressed through puberty normally and had regular menses. Biochemical results were in line with published data except for anti-Müllerian hormone (AMH) levels, which were lower compared with an age-matched reference group. AMH is an age-dependent hormone in pre-menopausal women and declines with age as follicle numbers fall. Nine patients not recruited into the study were known to have had 11 live births.[22]

Developmental toxicity studies

No premature deliveries or malformations were reported in 11 children fathered by seven patients having received ifosfamide in multidrug therapy.[9]

Mir et al. (2012) reported on 11 cases with high-grade sarcomas diagnosed during the third trimester of pregnancy and receiving chemotherapy until delivery. Five of these cases were retrieved from medical records from their own institute. These patients were treated with doxorubicin and ifosfamide. Of the remaining six cases, which were obtained from literature, two received a similar treatment while the other four had received ifosfamide in combination with several other chemotherapy agents. Abnormalities during pregnancy included, amongst others, oligo/anhydramnios (5/11), neutropenia (5/11) and intrauterine growth retardation (4/11). All deliveries were premature; six were by Caesarean section. One (female) newborn, delivered by emergency Caesarean section at 29 weeks had a birth weight of 720 g, Apgar scores of 3 and 7 at one and five minutes, anuria and intraventricular haemorrhage, and died at day 7. Another one

* All dose conversions in this study based on a bw of 31 kg and a body surface of 1.09 m^2 for a nine-year-old boy.[15]

(male), submitted to neonatal intensive care, had anaemia and thrombopenia at day 8, and was 'normal' at 12 weeks. Seven other babies had Apgar scores ≥7 while no data were available for the two remaining cases. All these nine babies appeared 'normal' at follow-up examination.[10]

The Committee notes that this may be a selected case series, as population-based denominators for exposure and effects were not available.

Lactation

No studies are available regarding ifosfamide levels in breast milk or the effects of ifosfamide on or via human lactation.

2.3 Animal studies

Fertility and developmental toxicity studies in laboratory animals are summarized in Annex F.

Fertility studies

In a preliminary experiment, Nagaoka et al. administered daily ifosfamide doses of 0, 5, 10 and 15 mg/kg bw to male Sprague-Dawley rats (n=10/group) intravenously for three weeks before mating and during mating and to female Sprague-Dawley rats (n=10/group) for three weeks before mating, during mating and until gestational day seven. Females were sacrificed at gestational day 14. No clear signs of toxicity were observed in the animals treated but body weight gain was decreased in the two higher dose groups. There were no statistically significant differences in mating and pregnancy rates between the groups, but treatment caused dose-related, statistically significant increases in embryolethality (see also below *Developmental toxicity studies*).[14]

Based on these results (and those of a subchronic toxicity study), Nagaoka et al. intravenously administered daily ifosfamide doses of 0, 1.25, 2.5 and 5 mg/kg bw to male Sprague-Dawley rats (n=20/group) from nine weeks before cohabitation and during cohabitation and to female rats (n=20/group) for two weeks before cohabitation, during habitation and until gestational day 7. During treatment, body weight and food and water consumption recordings and general condition observations were regularly made. Female animals were sacrificed at gestational day 20, and uterine contents were examined and maternal primary organs were weighed.

Treatment did not affect the percentage of males and females mating, the percentage of pregnant females, the number of corpora lutea, the number of implantation sites and the implantation ratio, and absolute or relative testis or ovary weights. There was no effect on male body weights while body weights of females given 2.5 or 5 mg/kg bw/day were decreased during gestation (no statistical analysis presented). At autopsy, male relative spleen weights were decreased at 1.25 mg/kg bw ($p<0.05$) and relative and absolute spleen weights at 2.5 and 5 mg/kg bw (all $p<0.01$). Male relative and absolute lung weights were increased at 5 mg/kg bw (both $p<0.05$). In females, body weights were decreased at 5 mg/kg bw ($p<0.05$), relative spleen weights at 2.5 mg/kg bw ($p<0.05$) and absolute spleen weights at 2.5 and 5 mg/kg bw (both $p<0.01$), while relative lung weights were increased at 5 mg/kg bw ($p<0.01$).[14]

Quinto et al. investigated the effect of ifosfamide on sperm cells. Male mice of genotype (C3H x C57BL/6)F1 (n=6), 11-15 weeks old, were administered intraperitoneal ifosfamide doses of 0, 12.5, 25, 50 or 100 mg/kg bw/day in distilled water for five consecutive days. Mice were killed 35 days after the first injection. Testis weights, sperm count and sperm morphology were evaluated.

No effect on sperm count was observed. The only effects observed were decreased testis weights ($p<0.05$) and increased sperm abnormalities ($p<0.05$; type not specified) at 100 mg/kg bw/day.[18]

Ypsilantis et al. studied the effect of ifosfamide on testes and semen characteristics in rabbits. Six-month-old male New Zealand white rabbits (n=10/group) were given single intravenous ifosfamide doses of 0, 60, 90, 120 or 240 mg/kg bw in distilled water. Semen was collected weekly, three weeks prior to treatment until the day of sacrifice, and examined for volume, sperm concentration and total sperm count. Sperm motility and morphology was only examined in the first ejaculate. Five rabbits per group were sacrificed one week and the remaining five 18 weeks after administration. Testes, epididymides and accessory sex glands were weighed. Testicular preparations were examined histologically.

Paired testis weights were – not dose relatedly – decreased at 90, 120 and 240 mg/kg bw, while accessory sex gland weights were decreased at 240 mg/kg bw one week after administration. Eighteen weeks after administration, no effects were observed on reproductive organ weights. No effects were observed on seminiferous tubule diameter, percentage of the most advanced germ cell type in seminiferous tubule cross section or number of germ cells per stage 1 seminiferous tubule cross section at post-treatment weeks 1 or 18. Total sperm

counts were decreased at post-treatment week 5 at 60, 90 and 120 mg/kg bw and from post-treatment weeks 5 to 7 at 240 mg/kg bw when compared to pre-treatment values ($p<0.05$). Primary sperm defects (i.e. small, double or deformed heads; swollen, abaxial or double midpieces; proximal droplets; double or coiled tails) were increased at post-treatment week 2 and 3 at 240 mg/kg bw ($p<0.05$), while secondary sperm defects (i.e. flagging or detached heads; bent, flagging or detached midpieces; distal droplets; bent tails) were increased at the 1st, 2nd and 6th week post-treatment at 120 mg/kg bw and from post-treatment weeks 3 to 7 at 240 mg/kg bw ($p<0.05$). Sperm motility was decreased from post-treatment weeks 2 to 5 at 240 mg/kg bw ($p<0.05$). The changes noted in sperm defects for each treatment group were higher than the corresponding changes in the control group over time, while changes in sperm count and motility were similar.[23]

Ehling et al. (1998) performed dominant lethal mutation tests in 13-14-week-old (102/E1xC3H/E1)F1 male mice following a standard protocol. Groups of mice were given single intraperitoneal injections of ifosfamide doses of 0, 300 and 600 mg/kg bw (n=40/group) or of 0, 250 and 600 mg/kg bw (n=35/group). After successful matings, females were sacrificed at gestational day 14-17, and uterine contents were inspected for the number of corpora lutea, preimplantation loss, and the number of total, live and dead implantations.

Apart from increased percentages of dead implants, no statistically significant changes were found in the aforementioned end points. Ifosfamide induced dominant lethal mutations in spermatozoa at doses of 300 and 600 mg/kg bw and in spermatids and spermatocytes at 600 mg/kg bw.[4]

Ehling et al. also conducted specific-locus mutation assays by treating 9-16-week-old mice with single intraperitoneal doses of 600 mg/kg bw (number of animals treated not presented) followed by sequential mating with 10-13-week-old untreated test-stock virgin females.

Ifosfamide induced specific-locus mutations in post-spermatogonial germ-cell stages but not in spermatogonial stem cells.[4]

Developmental toxicity studies

In the afore-mentioned preliminary study of Nagaoka et al., intravenous injection of doses of ifosfamide of 0, 5, 10 and 15 mg/kg bw into male Sprague-Dawley rats (n=10/group) for three weeks before mating and during mating and into female Sprague-Dawley rats (n=10/group) for three weeks before mating, during mating and until gestational day 7, caused increased embryolethality of 20%

(p<0.05), 86% (p<0.01) and 100% (p<0.01) at 5, 10 and 15 mg/kg bw, respectively, when compared to controls (4%). Maternal body weights were decreased at the two higher doses.[14]

In the main study in which intravenous doses of ifosfamide of 0, 1.25, 2.5 and 5 mg/kg bw were given to groups of 20 male rats from nine weeks before cohabitation and during cohabitation and to 20 female rats two weeks before cohabitation, during habitation and until gestational day 7, decreases were observed concerning foetal viability (at 2.5 and 5 mg/kg bw; 91 and 78% vs. 96% in controls; p<0.05 and <0.01, respectively), the number of living foetuses per pregnant rat (at 5 mg/kg bw; 11.3 vs. 13.3 in controls; p<0.05) and mean foetal body weight (at 5 mg/kg bw; 3.13±0.25 g vs. 3.50±0.19 in controls; p<0.05). Treatment did not cause increases in the number of foetuses with visceral or skeletal abnormalities. Maternal toxicity included decreased body weights at 5 mg/kg bw (p<0.05), decreased relative spleen weights at 2.5 mg/kg bw (p<0.05) and absolute spleen weights at 2.5 and 5 mg/kg bw (both p<0.01) and increased relative lung weights were increased at 5 mg/kg bw (p<0.01).[14]

In preliminary experiments, Nagaoka and Narama exposed groups of 10-13 female Sprague-Dawley rats to intravenous ifosfamide doses of 1.25, 2.5, 5 and 10 mg/kg bw from gestational day 7-17. Animals were sacrificed at gestational day 20. Increased foetal mortality (39 and 100% at 5 and 10 mg/kg bw, respectively) and decreased foetal body weights (at 2.5 and 5 mg/kg bw) were observed, but no external abnormalities. Treatment did not induce clear symptoms of toxicity but maternal body weights were decreased in the late stages of gestation.[12]

In the main study, groups of 30 pregnant rats were treated with intravenous doses of ifosfamide of 0, 1.25, 2.5 and 5 mg/kg bw/day from gestational day 7-17. Body weight and food and water consumption recordings and general condition observations were regularly made. Twenty animals/group were autopsied on gestational day 20 and uterine contents were examined and primary organs weighed. The remaining ten animals/group were allowed to litter and sacrificed at postnatal day 21. Pups were observed for up to 77 days (function, motor coordination, behaviour, fecundity by mating to produce an F2 generation).

No clear symptoms of toxicity were observed in the pregnant rats. Maternal body weights during gestation were decreased in the animals injected with 2.5 and 5 mg/kg bw compared to those in controls; in the postnatal period, the body weights of the high-dose animals were increased. Food and water consumption did not differ between groups during gestation but were decreased in the high-

dose animals from postnatal days 10-14 onwards. At autopsy on gestational day 20, dose-response related changes in absolute weights of the heart (increase; at 5 mg/kg bw: $p<0.05$), spleen (decrease; at 5 mg/kg bw: $p<0.05$) and the liver (decrease: at 2.5 mg/kg bw: $p<0.05$; at 5 mg/kg bw: $p<0.01$) and in relative weights of the heart (increase; at 2.5 and 5 mg/kg bw: $p<0.01$), the lung (increase; at 5 mg/kg bw: $p<0.01$) and the kidneys (increase; at 5 mg/kg bw: $p<0.01$) were seen. At autopsy on postnatal day 21, organ weight changes observed were increased absolute heart ($p<0.05$), increased absolute ovary ($p<0.05$) and decreased relative liver weights ($p<0.01$) all in animals treated with 5 mg/kg bw.

Treatment did not affect the number of corpora lutea, the number of corpora lutea/litter, the number of implantations, the number of implantations/litter and the sex ratio. At 5 mg/kg bw, the numbers of placental remnants, early, late and total deaths and foetal deaths/implantations were increased (all $p<0.01$) and the number of live foetuses/litter decreased ($p<0.01$). Mean body weights, body lengths, tail lengths and placental weights were decreased at all doses ($p<0.01$). Treatment with 5 mg/kg bw caused an increase in the number of foetuses with external abnormalities (4/169 (2.4% vs. 0/265 in controls; $p<0.05$) and in the number of litters with foetuses with external abnormalities (3 vs. 0; n.s. (not statistically significant)). Skeletal effects observed included increased numbers of foetuses with cervical rib (1.25 mg/kg bw: 10/22 ($p<0.01$); 2.5 mg/kg bw: 7/32 (n.s.); 5 mg/kg bw: 6/19 ($p<0.05$; controls: 1/21). At all doses, there were signs of delayed ossification.

As to the groups that were allowed to deliver, there was no effect on gestational period. The total number of implantations was dose-relatedly decreased as was the number of live pups (both n.s.). At 5 mg/kg bw, the number of live pups/litter, the rate of birth and the stillborn rate, body weights of male and female pups (at birth, four-day old and one-, two- and three-week old) and viability, when four-day and three-week old, were decreased and the number of pups with skeletal abnormalities increased (all $p<0.01$). At post-mortem examinations of four-day-old pups, no external abnormalities were seen. Recording of primary organs showed decreases in absolute weights of almost all these organs ($p<0.05$) and in absolute and relative weights of testis $p<0.01$) in high-dose males and of absolute weights of lungs ($p<0.05$) and testes ($p<0.01$) in mid-dose males and increases in relative heart weights ($p<0.05$) in mid-dose females. In three-week-old pups, there were increases in the number of pups with skeletal abnormalities at 5 mg/kg bw (1/7 (15%) vs. 0/58 in controls; $p<0.01$), in the number of pups with cervical ribs at 2.5 and 5 mg/kg bw (6/59 (10%) and 1/7 (15%), respectively, vs. 0/58; $p<0.05$ and 0.01, respectively). In seven-week-old

pups, absolute weights of all organs recorded were decreased in high-dose male pups (n=3; $p<0.01$) and of brain ($p<0.05$), spleen ($p<0.01$) and kidneys ($p<0.05$) in high-dose females (n=2). In these females, relative ovary weights were increased ($p<0.01$). Postnatal functional, behavioural and sexual development was generally not affected, apart from a dose-related impaired performance in the water multiple T-maze test in six-weeks-old pups (at 5 mg/kg bw: $p<0.05$) and decreased gestational periods in pregnant F1 offspring at 1.25 and 5 mg/kg bw ($p<0.05$).[12]

Nagaoka and Narama also tested the effects of single intravenous doses of ifosfamide of 0, 5 and 10 mg/kg bw administered on gestational day 7, 10 or 13 (n=10/group). Injection of 10 mg/kg bw on gestational day 7 caused increases in the number of foetal deaths ($p<0.05$) and decreases in mean live foetal mean body weights, body lengths and tail lengths (all $p<0.01$) and of placental weights ($p<0.05$); injection of 5 mg/kg bw also induced decreased body weights ($p<0.05$), body lengths ($p<0.01$) and tail lengths ($p<0.05$).

Injection of 10 mg/kg bw on gestational day 10 resulted in similar effects as seen when given at gestational 7 and further increases in the number of foetuses and litters with external, visceral and skeletal abnormalities ($p<0.01$); after injection of 5 mg/kg bw, decreases in mean body weights, tail lengths and placental weights and increases in the number of foetuses with external and skeletal abnormalities were reported (all $p<0.01$).

Injection of 10 mg/kg bw on gestational day 13 caused decreased placental weights ($p<0.01$), increased numbers of foetuses with external, visceral and skeletal abnormalities ($p<0.01$) and increased numbers of litters with visceral and skeletal abnormalities ($p<0.05$) while 5 mg/kg bw increased the number of foetuses and litters with skeletal abnormalities ($p<0.01$).[12]

Nagaoka et al. administered intravenous doses of 0, 2.5, 5 and 10 mg/kg bw to groups of 20 rats from gestational day 17 to postnatal day 21. Thereafter, dams were sacrificed and organ weights recorded. The offspring as examined for external and internal abnormalities, for effects on organ weights, development, and behaviour and reproductive performance.

Treatment did not affect maternal body weights but absolute and relative spleen weights were decreased at all doses ($p<0.01$) and absolute and relative liver weights at 5 mg/kg bw ($p<0.05$ and <0.01, respectively).

No statistically significant differences between groups were observed with respect to length of the gestational period, the total number of implantations, the parturition index, the number of live pups, live litter size, percentage of viability

for four days and the weaning rate but the number of stillborns was decreased at 5 ($p<0.05$) and 10 mg/kg bw ($p<0.01$). No external abnormalities were seen.

Male pup birth weights and male and female pup birth weights were decreased at 5 and 10 mg/kg bw, respectively ($p<0.05$, <0.01 and <0.01, respectively), and body weights of male and female pups of the high-dose group continued to be lower than those of controls throughout the lactational period (p mostly <0.01). Examination of effects on development, puberty, reflexes and behavioural tests showed retarded auricle separation at 5 and 10 mg/kg bw (both $p<0.01$), increased ambulation and rearing in the open-field test (both $p<0.01$) and an increased number of errors on the third day in the water T-maze test ($p<0.05$). Upon sacrifice just after weaning, no consistent effects on organ weights were seen except for increased relative male and female lung weights ($p<0.01$). Examination of seven-week-old pups showed increases in the number of animals with external and visceral abnormalities (mainly hydrocephalus) and in the number of litters having rats with external and visceral abnormalities (all $p<0.01$). Upon sacrifice, no consistent changes were observed in relative organ weights while absolute weights of the heart, lungs, spleen, kidneys and liver in males and females and of the testes were statistically significantly decreased at 10 mg/kg bw. Mating of groups of 17-20 animals/sex within the same dose groups did not reveal consistent effects of ifosfamide treatment of maternal animals on the reproductive performance of the F1 generation and the development of the F2 generation.[13]

Nagaoka and Narama initially treated groups of five pregnant rabbits with intravenous doses of ifosfamide of 0, 5, 10, 30 and 50 mg/kg bw from gestational days 6-18. Animals were sacrificed at gestational day 29. At 50 mg/kg bw, all animals died. At 30 mg/kg bw, body weights were decreased and foetal mortality was 100%. In the two lower dose groups, foetal mortality was ‘mildly’ increased.

Based on these results, Nagaoka and Narama treated groups of ten pregnant rabbits with intravenous doses of ifosfamide of 0, 5, 10 and 20 mg/kg bw/day from gestational days 6-18. Body weight and food and water consumption recordings and general condition observations were performed regularly. Dams were sacrificed on gestational day 29 and uterine contents were examined.

Treatment did not affect maternal body weight gain or water or food consumption when compared to controls. At 20 mg/kg bw, 2/10 rabbits had anaemia. At autopsy, the only consistent effect on absolute and/or relative organ weights were dose-related increases in spleen weights being statistically significantly different ($p<0.05$) at 5 and 10 mg/kg bw.

Apart from decreased numbers of corpora lutea/litter (10.1±0.9 vs. 12.9±1.7 in controls; $p<0.01$) and decreased mean foetal body weights (30.9±0.5 g vs. 37.1±5.4 g in controls; $p<0.01$) at 20 mg/kg bw, no statistically significant changes were seen in the number of implantations, the number of implantations/ litter, the number of foetal deaths (early and late death, placental remnants, total), the number of foetal deaths/litter, number of live foetuses, the number of live foetuses/liter, sex ratio, mean body length, mean placental weight and the viability of live foetuses at six and 24 hours. Treatment with 20 mg/kg bw caused increased numbers of foetuses with external malformations, viz. ectrodactyla (7/82 (9%) vs. 0/98 in controls; $p<0.01$) of litters with external malformations (4 vs. 0; n.s.) of foetuses with skeletal abnormalities (22/82 (27%) vs. 1/98 (1%); $p<0.01$) of litters with foetuses with skeletal abnormalities (7 vs. 1; $p<0.01$) and of foetuses with skeletal variations (62/82 (76%) vs. 39/98 (40%); $p<0.01$). Apart from one case of ectrodactyla at 10 mg/kg bw, no effects were observed at doses of 5 or 10 mg/kg bw.[11]

Bus and Gibson investigated the teratogenicity of ifosfamide with the knowledge that the analogue cyclophosphamide is teratogenic. Female Swiss Webster mice (n=6-7/group) were administered intraperitoneal doses of ifosfamide of 0, 5, 10 or 20 mg/kg bw in saline on gestational day 11. Gravid females were sacrificed on gestational day 19. The number of live, dead and resorbed foetuses, foetal weights and gross abnormalities were recorded. Half of the litters was examined for soft tissue abnormalities and the remaining half for skeletal abnormalities.

In the control group, no gross, soft tissue or skeletal abnormalities were observed. At 5 mg/kg bw, the percentages of supernumerary ribs (55±14%; $p<0.05$) and of absent or not ossified phalanges (15±10.5% vs. 0% in controls; n.s.) were increased. At 10 mg/kg bw, foetal weights and crown-rump lengths were decreased ($p<0.05$). Gross abnormalities observed included adactyly (16±14%; n.s.). With respect to soft tissue and skeletal abnormalities, there were increased rates of amongst others cleft palate (9±4%; n.s.), internal hydrocephalus (10±5%; n.s.), cryptorchidism (21±16%; n.s.), fused sternebrae (22±11%; n.s.), supernumerary ribs (10±8%; n.s.), fused vertebrae (50±16%; $p<0.05$), absent or not ossified metatarsals (25±13%; n.s.), metacarpals (25±13%; n.s.) and phalanges (32±15%; n.s.). At 20 mg/kg bw, the number of foetuses, foetal weights and crown-rump lengths were decreased (all: $p<0.05$) and the number of resorptions increased ($p<0.05$). The foetuses showed increased rates of open eyes (90±10%), external hydrocephalus (90±10%), micromelia (63±19%), adactyly (83±17%), syndactyly (65±16%), microcaudate (78±15%), and kinky tails (27±19%) (all: $p<0.05$). Soft tissue examination

revealed statistically significant ($p<0.05$) increases in the rates of internal hydrocephalus (90±10%), microphakia (47±23%), kidney ectopia (67±21%) and hydronephrosis (77±14.5%) and non-significant increases of cleft palate (20±20%), exencephaly (10±10%), and cryptorchidism (40±24.5%). As to skeletal abnormalities, incidences of defects such as absent or not ossified skull bones, sternebrae, fibula, ulna, radius, metatarsals, metacarpals, and phalanges, and fused sternebrae, ribs and vertebrae were increased ($p<0.05$), ranging from 44 (fused sternebrae) to 100% (fused ribs/vertebrae; absent or not ossified fibula). No information of toxic effects on dams was given.[2]

Weigt et al. investigated exposure of zebra fish (Danio rerio) embryos to ifosfamide concentrations of 0.25 to 4 mM. Embryos were scored for developmental effects and lethality on post-fertilization days 1, 2 and 3. The numbers of embryos with developmental effects and of dead embryos were statistically significantly increased at concentrations of 1 mM ifosfamide and higher. Malformations of the chorda, head, sacculi/otoliths and tail (tip) and growth retardation were observed. Several of these developmental effects could be detected from two days post fertilization. The authors compared the EC_{20} of 916 µM to the human plasma concentration of ~7.6-490 µM after ifosfamide treatment in the absence of umbilical cord blood data showing that teratogenic effects in zebra fish embryos occur in the same range as human plasma concentrations.[21]

Lactation

No studies were available regarding ifosfamide levels in animal milk.

Nagaoka et al. administered intravenous doses of 0, 2.5, 5 and 10 mg/kg bw to groups of 20 rats from gestational day 17 to postnatal day 21. Body weights of male and female pups of the high-dose group were decreased throughout the lactational period (p mostly <0.01). Examination of effects on development, puberty, reflexes and behavioural tests showed some effects on developmental and behavioural end points. Upon sacrifice just after weaning, no consistent effects on organ weights were seen except for increased relative male and female lung weights ($p<0.01$).[13]

2.4 Conclusions

The Committee notes that the doses that caused adverse effects in animals were far lower than the human therapeutic doses. However, this does not affect the

following conclusions and classification proposals since these are based on hazard evaluation only.

Fertility

No effects were observed in semen and biochemical analyses in male patients treated for cancer according to multidrug protocols including ifosfamide.[5] In a study in which groups of male patients treated with combinations of drugs with and without ifosfamide were compared, the incidence of aazospermia was increased in the groups receiving ifosfamide.[9] In two studies among groups of male patients receiving combinations of drugs with ifosfamide as the only alkylating/gonadotoxic drug, elevated LH levels (semen characteristics not investigated)[19], decreased sperm counts, increased FSH levels and decreased inhibin B levels[22] were seen. In view of the small group sizes and the inconsistent results, the Committee is of the opinion that the human studies are not sufficient for classification.

In laboratory animals, ifosfamide did not affect fertility end points in a study in which male and female rats were intravenously injected from two weeks before starting cohabituation, during habituation and until gestational day 7.[14] In mice, ifosfamide caused decreased testes weights and increased sperm abnormalities following intraperitoneal administration for five days[18] and dominant lethal and specific-locus mutations following single intraperitoneal injections[4]. In rabbits, transient decreases in testis and accessory gland weights, sperm counts and sperm motility, and sperm defects were seen following a single intravenous injection.[23]

Based on animal data, the Committee proposes to classify ifosfamide for effects on fertility in category 1B.

Developmental toxicity

Only two reports on developmental effects in humans were available: one small study on developmental toxicity in children fathered by chemotherapy patients having received ifosfamide[9] and one case series on the outcome of pregnancies of women with high-grade sarcomas diagnosed during the third trimester of pregnancy and receiving chemotherapy with ifosfamide in combination with several other agents until delivery[10].

The Committee is of the opinion that this information is not sufficient for classification.

Ifosfamide intravenously administered to rats before and during cohabitation and the subsequent first seven gestational days, from gestational day 7-17 and from gestational day 17 to postnatal day 21 induced prenatal and postnatal effects including increased foetal mortality, decreased foetal growth, increased frequency of external and visceral abnormalities (among which hydrocephalus) and skeletal defects (abnormalities, variations, reduced ossification) and impaired performance in behavioural tests[12-14], and, injected into rabbits from gestational day 6-18, increased incidences of external and skeletal abnormalities (among which ectrodactyla)[11]. In these studies, maternal toxicity, viz. decreased body weights, decreased spleen and increased lung weights in rats[12-14] and decreased spleen weights in rabbits[11], were reported. However, in one of these studies in rats[12], retarded ossification was seen in the absence of maternal toxicity. In addition, intravenous injection on gestational day 7, 10 or 13 into rats[12] and intraperitoneal injection on gestational day 11 into mice[2] resulted in similar developmental effects.

The Committee is of the opinion that the developmental effects occurred independently from maternal toxicity. Therefore, based on the animal data, the Committee recommends to classify ifosfamide in category 1B.

Lactation

No human or animal data were available on the excretion of ifosfamide in human or animal milk nor human data on the effects of ifosfamide on or via lactation.

In rats, intravenous injection of ifosfamide from gestational day 17 to postnatal day 21 caused decreased pup body weights as well as some effects on developmental and behavioural end points.[13] The effects seen could be caused by exposure during lactation, by prenatal exposure through the mother or both, but the available data do not allow the Committee to distinguish between these possibilities.

Therefore, the Committee concluded that a lack of appropriate data precludes assessment of ifosfamide for effects on or via lactation.

Proposed classification for fertility

Category 1B, H360F.

Proposed classification for developmental toxicity

Category 1B, H360D.

Proposed labelling for effect on or via lactation

Lack of appropriate data precludes the assessment of ifosfamide for effects on or via lactation.

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A	The Committee
B	The submission letter (in English)
C	Comments on the public draft
D	Regulation (EC) 1272/2008 of the European Community
E	Additional considerations to Regulation (EC) 1272/2008
F	Fertility and developmental toxicity studies

Annexes

Annex **A**

The Committee

- A.H. Piersma, *Chairman*
  Professor of Reproductive and Developmental Toxicology, Utrecht University, Utrecht and National Institute of Public Health and the Environment, Bilthoven
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- A.S.A.M. van der Burght, *Scientific Secretary*
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- J.T.J. Stouten, *Scientific Secretary*
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The first draft of this report was prepared by Dr. H.M. Barentsen, from the Regulatory Affairs Department of WIL Research Europe BV (Den Bosch,

the Netherlands) by contract with the Ministry of Social Affairs and Employment.

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 chairperson 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 inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *Ifosfamide*
Your reference : DGV/MBO/U-932542
Our reference : U-8079/HS/cn/543-K14
Enclosed : 1
Date : April 3, 2014

Dear Minister,

I hereby submit the advisory report on the effects of ifosfamide on fertility and on the development of the progeny; it also concerns effects on lactation and on the progeny via lactation. This advisory report is part of an extensive series in which reproduction toxic substances are classified in accordance with European guidelines. This involves substances to which people may be exposed occupationally.

The advisory report was prepared by a permanent committee of the Health Council of the Netherlands, the Subcommittee on the Classification of Reproduction Toxic Substances. The advisory report was consequently reviewed by the Health Council's Standing Committee on Health and the Environment.

Today I sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their information.

Yours sincerely,
(signed)
Prof. dr. W.A. van Gool,
President

Annex C

Comments on the public draft

A draft of the present report was released in 2013 for public review. The following organisation and persons have commented on the draft document:

- T.J. Lentz, D. Murray, S. Rengasamy, K. Krajnak, C. B'Hymer; National Institute for Occupational Safety and Health, Cincinnati OH, USA

The received comments, and the reply by the Committee can be found on the website of the Health Council.

Annex **D**

Regulation (EC) 1272/2008 of the European Community

3.7 Reproductive toxicity

3.7.1 Definitions and general considerations

3.7.1.1 Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. The definitions presented below are adapted from those agreed as working definitions in IPCS/EHC Document No 225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. For classification purposes, the known induction of genetically based heritable effects in the offspring is addressed in Germ Cell Mutagenicity (section 3.5), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

In this classification system, reproductive toxicity is subdivided under two main headings:

(a) adverse effects on sexual function and fertility;

(b) adverse effects on development of the offspring.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nonetheless, substances with these effects, or mixtures containing them, shall be classified as reproductive toxicants.

3.7.1.2 For the purpose of classification the hazard class Reproductive Toxicity is differentiated into:

- adverse effects
  - on sexual function and fertility, or
  - on development;
- effects on or via lactation.

3.7.1.3 Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

3.7.1.4 Adverse effects on development of the offspring

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

3.7.1.5 Adverse effects on or via lactation are also included in reproductive toxicity, but for classification purposes, such effects are treated separately (see Table 3.7.1 (b)). This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

3.7.2 Classification criteria for substances

3.7.2.1 Hazard categories

3.7.2.1.1 For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Table 3.7.1(a) Hazard categories for reproductive toxicants.

Categories		Criteria
CATEGORY 1		Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
	Category 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
	Category 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
CATEGORY 2		Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Table 3.7.1(b) Hazard category for lactation effects.

EFFECTS ON OR VIA LACTATION
Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to 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, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

3.7.2.2 Basis of classification

3.7.2.2.1 Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence (see 1.1.1). Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

The classification of a substance is derived from the hazard categories in the following order of precedence: Category 1A, Category 1B, Category 2 and the additional Category for effects on or via lactation. If a substance meets the criteria for classification into both of the main categories (for example Category 1B for effects on sexual function and fertility and also Category 2 for development) then both hazard differentiations shall be communicated by the respective hazard statements. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into Category 1A, Category 1B or Category 2.

3.7.2.2.2 In the evaluation of toxic effects on the developing offspring, it is important to consider the possible influence of maternal toxicity (see section 3.7.2.4).

3.7.2.2.3 For human evidence to provide the primary basis for a Category 1A classification there must be reliable evidence of an adverse effect on reproduction in humans. Evidence used for classification shall ideally be from well conducted epidemiological studies which include the use of appropriate controls, balanced assessment, and due consideration of bias or confounding factors. Less rigorous data from studies in humans shall be supplemented with adequate data from studies in experimental animals and classification in Category 1B shall be considered.

3.7.2.3 Weight of evidence

3.7.2.3.1 Classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence, see section 1.1.1. This means that all available information that bears on the determination of reproductive toxicity is considered together, such as epidemiological studies and case reports in humans and specific reproduction studies along with sub-chronic, chronic and special study results in animals that provide relevant information regarding toxicity to reproductive and related endocrine organs. Evaluation of substances chemically related to the substance under study may also be included, particularly when information on the substance is scarce. The weight given to the available evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, the presence of maternal toxicity in experimental animal studies, level of statistical significance for inter-group differences, number of endpoints affected, relevance of route of administration to humans and freedom from bias. Both positive and negative results are assembled together into a weight of evidence determination. A single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification (see also 3.7.2.2.3).

3.7.2.3.2 Toxicokinetic studies in animals and humans, site of action and mechanism or mode of action study results may provide relevant information which reduces or increases concerns about the hazard to human health. If it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified.

3.7.2.3.3 If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments.

3.7.2.3.4 Data from animal studies ideally shall provide clear evidence of specific reproductive toxicity in the absence of other systemic toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects shall be assessed to the extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternally toxic doses shall not be automatically discounted. Discounting devel-

opmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis when a causal relationship is established or refuted.

3.7.2.3.5 If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. In some situations it can be assumed that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, if the substance is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups; or they are prostrate or dying.

3.7.2.4 Maternal toxicity

3.7.2.4.1 Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms. In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification.

3.7.2.4.2 Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.

3.7.2.4.3 Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

3.7.2.4.4 Some of the end points used to assess maternal effects are provided below. Data on these end points, if available, need to be evaluated in light of their statistical or biological significance and dose response relationship.

Maternal mortality:

an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.

Mating index

(no. animals with seminal plugs or sperm/no. mated × 100) (*)

Fertility index

(no. animals with implants/no. of matings × 100)

Gestation length

(if allowed to deliver)

Body weight and body weight change:

Consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available. The calcula-

* () It is recognised that the Mating index and the Fertility index can also be affected by the male.

tion of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

Food and water consumption (if relevant):

The observation of a significant decrease in the average food or water consumption in treated dams compared to the control group is useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption need to be evaluated in conjunction with maternal body weights when determining if the effects noted are reflective of maternal toxicity or more simply, unpalatability of the test material in feed or water.

Clinical evaluations (including clinical signs, markers, haematology and clinical chemistry studies):

The observation of increased incidence of significant clinical signs of toxicity in treated dams relative to the control group is useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree and duration of clinical signs shall be reported in the study. Clinical signs of maternal intoxication include: coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.

Post-mortem data:

Increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data, including absolute organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared to those in the control group, may be considered evidence of maternal toxicity.

3.7.2.5 Animal and experimental data

3.7.2.5.1 A number of internationally accepted test methods are available; these include methods for developmental toxicity testing (e.g. OECD Test Guideline 414), and methods for one or two-generation toxicity testing (e.g. OECD Test Guidelines 415, 416).

3.7.2.5.2 Results obtained from Screening Tests (e.g. OECD Guidelines 421 — Reproduction/ Developmental Toxicity Screening Test, and 422 — Combined Repeated Dose Toxicity Study with

Reproduction/Development Toxicity Screening Test) can also be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

3.7.2.5.3 Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads.

3.7.2.5.4 Evidence from in vitro assays, or non-mammalian tests, and from analogous substances using structure-activity relationship (SAR), can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. Inadequate data shall not be used as a primary support for classification.

3.7.2.5.5 It is preferable that animal studies are conducted using appropriate routes of administration which relate to the potential route of human exposure. However, in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity. However, if it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals shall not be classified.

3.7.2.5.6 Studies involving routes of administration such as intravenous or intraperitoneal injection, which result in exposure of the reproductive organs to unrealistically high levels of the test substance, or elicit local damage to the reproductive organs, including irritation, must be interpreted with extreme caution and on their own are not normally the basis for classification.

3.7.2.5.7 There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model.

3.7.2.5.8 In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would

not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area.

3.7.2.5.9 However, specification of the actual 'limit dose' will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1 000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

3.7.3 Classification criteria for mixtures

3.7.3.1 Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.7.3.1.1 The mixture shall be classified as a reproductive toxicant when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 reproductive toxicant and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for Category 1A, Category 1B and Category 2 respectively.

3.7.3.1.2 The mixture shall be classified for effects on or via lactation when at least one ingredient has been classified for effects on or via lactation and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for the additional category for effects on or via lactation.

Table 3.7.2 Generic concentration limits of ingredients of a mixture classified as reproduction toxicants or foreffects on or via lactation that trigger classification of the mixture.

Ingredient classified as:	Generic concentration limits triggering classification of a mixture as:			
	Category 1A reproductive toxicant	Category 1B reproductive toxicant	Category 2 reproductive toxicant	Additional category for effects on or via l actation
Category 1A reproductive toxicant	≥ 0,3 % [Note 1]			
Category 1B reproductive toxicant		≥ 0,3 % [Note 1]		
Category 2 reproductive toxicant			≥ 3,0 % [Note 1]	
Additional category for effects on or via lactation				≥ 0,3 % [Note 1]

Note The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).
Note 1 If a Category 1 or Category 2 reproductive toxicant or a substance classified for effects on or via lactation is present in the mixture as an ingredient at a concentration above 0,1 %, a SDS shall be available for the mixture upon request.

3.7.3.2 Classification of mixtures when data are available for the complete mixture

3.7.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients of the mixture. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual components. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of reproduction test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.7.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.7.3.3.1 Subject to paragraph 3.7.3.2.1, where the mixture itself has not been tested to determine its reproductive toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.7.4 Hazard Communication

3.7.4.1 Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.7.3

Table 3.7.3 Label elements for reproductive toxicity.

Classification	Category 1A or Category 1B	Category 2	Additional category for effects on or via lactation
GHS Pictograms			No pictogram
Signal Word	Danger	Warning	No signal word
Hazard Statement	H360: May damage fertility or the unborn child (state specific effect if known)(state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H362: May cause harm to breast-fed children.
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281	P201 P260 P263 P264 P270
Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

Annex **E**

Additional considerations to Regulation (EC) 1272/2008

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC)1272/2008) presented in Annex D. The classification of compounds 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 guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, 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 offspring, the compound will be classified in category 1A, irrespective of the general toxic effects (see Annex D, 3.7.2.2.1.)
- adverse effects in a reproductive study, occurring without reporting the parental or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in *general* toxicity studies
- clear adverse reproductive effects will not be disregarded on the basis of reversibility per se

- the Committee dot not only use guideline studies (studies performed according to OECD* standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

* Organisation for Economic Cooperation and Development.

Annex **F**

Fertility and developmental studies in animals

Table 1 Fertility studies with ifosfamide in animals.

authors	species	experimental period/ design	dose/route	general toxicity	effects on reproductive organs/ effects on reproduction
Nagaoka et al. (1982)	Sprague-Dawley rats (n=10/sex/ group	males: 3 wk before mating, during mating females: 3 wk before mating, during mating, until gd 7; sacrifice: gd 14	0, 5, 10, 15 mg/kg bw/d; iv	see below Table 2	preliminary study no effect on mating and pregnancy rates
Nagaoka et al. (1982)	Sprague-Dawley rats (n=20/sex/ group	males: 9 wk before cohabitation, during cohabitation females: 2 wk before cohabitation, during cohabitation,, until gd 7; sacrifice: gd 20; examination of uterine contents, weighing primary organs	0, 1.25, 2.5, 5 mg/kg bw/d; iv	see below Table 2	main study no effect on percentage of males and females mating; percentage of pregnant females; number of corpora lutea; number of implantation sites; implantation ratio; relative/absolute testis wt; relative/absolute ovary wt
Quinto et al. (1988)	male (C3H xC57BL/ 6)F_1 mice (n=6/ group) (11-15-wk old)	5 d; sacrifice: 35 d after 1st injection; testis wt, sperm count and morphology (500 spermatozoa/animal) evaluated	0, 12.5, 25, 50, 100 mg/kg bw/ d; ip	not reported	sperm count (n x 10^6/epididymis): 4.7±0.5, 2.5±0.2, 4.4±1.2, 2.0±0.5, 2.5±0.9, at 0, 12.5, 25, 50, 100 mg/ kg bw/d, respectively testis weight (mg/10 g bw): 76.9±1.6, 72.8±2.9, 74.7±2.5, 69.4±2.5, 62.4±1.8*, respectively sperm abnormalities (%): 1.6±0.14, 2.1±0.35, 1.6±0.17, 2.6±0.53, 3.9±0.60*, respectively

Ypsilantis et al. (2003)	male New Zealand white rabbits (n=10/group) (6-mo old)	single dose; sacrifice: n=5/group 1 wk and n=5/group 18 wk after injection; parameters evaluated: reproductive organ wt (testes, epididymides, accessory glands); testicular histology (seminiferous tubule diameter, percentage of the most advanced germ cell type in seminiferous tubule cross section, number of germ cells per stage 1 seminiferous tubule cross section); semen quality (weekly collection - 4 times on 1 d – for 3 wk prior to treatment until day of sacrifice: examined for volume, sperm concentration, total sperm count, sperm motility, sperm morphology); libido (willingness to mount a teaser doe in oestrus; ability to ejaculate)	0, 60, 90, 120, 240 mg/kg bw; iv	libido: no effect semen quality: total sperm count: decreased (vs. pre-treatment values; $p<0.05$;) at wk 5 post-treatment at 60, 90, 120 mg/kg bw and from wk 5-7 post-treatment at 240 mg/kg bw; sperm defects: primary defects (i.e. small, double, or deformed heads, swollen, abaxial, or double mid pieces, proximal droplets, and double or coiled tails): increased (vs. controls; $p<0.05$) at wk 2 and 3 post-treatment at 240 mg/kg bw; secondary defects (i.e. flagging or detached heads, bent, flagging or detached midpieces, distal droplets, and bent tails) increased (vs. controls; $p<0.05$) at wk 1, 2, 6 post-treatment at 120 mg/kg bw, at wk 3-7 at 240 mg/kg bw; sperm motility: decreased (vs. controls; $p<0.05$) at wk 2-5 post-treatment at 240 mg/kg bw. post-mortem: 1 wk post-treatment: bw: no effect; testis weight: decreased (vs. controls; $p<0.05$) paired testis weight at 90, 120 and 240 mg/kg bw (not dose related); no effect on left or right testis weight paired epididymis weight: no effect on head, tail or total wt accessory sex gland wt: decreased (vs. controls; $p<0.05$) at 240 mg/kg bw testicular histology: no effect 18 wk post-treatment: bw: no effect reproductive organ wt: no effect testicular histology: no effect

abbreviations: bw=body weight; d=day(s); ip=intraperitoneal; iv=intravenous; mo=month(s); wk=week(s); wt=weight(s).
*: $p<0.05$; **: $p<0.01$.

Table 2 Developmental toxicity studies with ifosfamide in animals.

authors	species	experimental period/ design	dose/ route	general toxicity	developmental toxicity
Nagaoka et al. (1982)	Sprague-Dawley rats (n=10/ sex/ group	see above Table 1	0, 5, 10, 15 mg/kg bw/ d; iv	no clear signs of toxicity; decreased bw gain at 10, 15 mg/kg bw	preliminary study embryolethality: 4%, 20%*, 86%**, 100%**
Nagaoka et al. (1982)	Sprague-Dawley rats (n=20/ sex/ group	see above Table 1	0, 1.25, 2.5, 5 mg/ kg bw/d; iv	male bw (g): 500±41, 494±28, 498±35, 495±39, female bw (g): 395±24, 397±34, 385±26, 377±24* male organ wt, absolute wt of lung (g): 1.71±0.16, 1.74±0.22, 1.73±0.17, 1.83±0.18*, of spleen (mg): 991±148, 901±145, 824±130**, 763±83**, relative wt of lung (mg/100 g bw): 337±27, 340±36, 336±30, 364±41*, of spleen (mg/100 g bw): 196±32, 176±26*, 159±18**, 151v14**; female organ wt, absolute wt of spleen (mg): 705±86, 714±116, 633±61**, 634±72**, relative wt of lung (mg/ 100 g bw): 344±36, 333±20, 355±42, 384±44**, of spleen 178±17, 180±25, 165±11*, 169±20, of liver (g/100 g bw): 3.88±0.23, 4.05±0.17*, 3.88±0.22, 3.99±0.19, of kidney (mg/100 g bw): 499±39, 516±41, 520±34, 544±67*	main study foetal viability (%): 96, 93, 91*, 78** number of living foetuses/pregnant rat: 13.3, 13.1, 11.8, 11.3* mean foetal bw (g): 3.50±0.19, 3.43±0.19, 3.42±0.23, 3.13±0.25* no increases in numbers of foetuses with visceral or skeletal abnormalities
Nagaoka/ Narama (1982)	female Sprague-Dawley rats (n=10-13/group)	gd 7-17; sacrifice: gd 20	0, 1.25, 2.5, 5, 10 mg/kg bw/ d; iv	no clear symptoms of toxicity; decreased bw in late stages of gestation	preliminary study increased foetal mortality at 5 and 10 mg/kg bw; 39 and 100%, resp. decreased foetal bw at 2.5 and 5 mg/kg bw
Nagaoka/ Narama (1982)	female Sprague-Dawley rats (n=30/ group)	gd 7-17; sacrifice: gd 20 (n=20/ group) examination of uterine contents, weighing primary organs pnd 21 (n=10/ group) pup observation for up to 77 d (function, motor coordination, behaviour,	0, 1.25, 2.5, 5 mg/ kg bw/d; iv	no clear signs of toxicity; decreased bw at 2.5 and 5 mg/kg bw during gestation; in postnatal period increased bw at 5 mg/kg bw; no effect on food, water consumption during gestation, decreased at 5 mg/ kg bw from pnd 10-14 onwards; at autopsy on gd 20: absolute wt of heart (mg): 924±99, 927±84, 957±82, 993±91* of spleen (mg): 631±73, 594±67, 601±81, 586±91* of liver (g): 14.53±1.32, 14.15±1.61, 13.66±1.27*, 12.65±1.43** relative wt of heart (mg/100 g bw): 238±20, 244±18, 258±17**, 291±17** of lung (mg/100 g bw): 344±40,	main study groups sacrificed on gd 20 no effect on number of corpora lutea; number of corpora lutea/ litter; number of implantations; number of implantations/litter; sex ratio parameters affected: number of early deaths: 13, 10, 11, 55** number of placental remnants: 2, 4, 1, 32** number of late death: 0, 1, 4, 26** number of total death: 15, 15, 16, 113** foetal death/implantations (%): 5.4, 5.2, 5.5, 40.1** mean number of live foetuses/litter:

fecundity by mating to produce F2 generation)		340±20, 365±30, 396±59** kidneys (mg/100 g bw): 485±28, 492±39, 500±57, 570±59** at autopsy on pnd 21: absolute wt of heart (g): 1.01±0.09, 1.01±0.08, 1.00±0.10, 1.17±0.11* of ovaries (mg): 90.1±7.5, 96.2±9.8, 103.6±29, 106.5±14.4* relative wt of liver (g/100 g bw): 4.25±0.20, 4.21±0.33, 3.89±0.50, 3.61±0.34**	13.3±2.0, 13.6±2.7, 13.8±2.8, 8.5±5.8** mean bw (g): 3.53 no clear signs of toxicity; decreased bw gain at 10, 15 mg/kg bw 0.22, 3.21±0.22**, 3.05±0.21, 1.84±0.34** mean body length (cm): 3.6±0.1, 3.5±0.1**, 3.5±0.1**.1, 2.9±0.2** mean tail length (cm): 1.4±0.1, 1.3±0.0**, 1.3±0.0**, 1.1±0.1** mean placental wt (g): 0.45±0.06, 0.38±0.05**, 0.32±0.03**, 0.16±0.04** number of foetuses with external abnormalities (%): 0/265, 0/272, 0/275, 4/169 (2.4* number of litters with foetuses with external abnormalities: 0, 0, 0, 3 number of foetuses with cervical rib: 1/21, 10/22**, 7/32, 6/19* groups allowed to deliver no effect on gestational period total number of implantations: 154, 146, 142, 133 number of live pups: 142, 133, 129, 60 mean number of live pups/litter: 14.2±1.1, 13.3±1.8, 12.9±3.0, 6.7±5.3** mean rate of birth (%): 92.5±6.7, 94.7±6.0, 91.8±7.1, 50.5±36.9 stillborn rate (%): 0, 3.6*, 1.5, 13.0** mean male pup bw (g): at birth: 6.2±0.5, 6.0±0.6, 5.7±0.4*, 3.7±0.4**; pnd 4: 9.0±0.8, 8.8±1.3, 8.8±1.1, 5.1±1.2**; pnd 7: 14.7±1.1, 14.0±2.3, 14.3±2.1, 8.2±0.7**; pnd 14: 29.9±1.3, 27.5±2.6*, 28.0±2.4*, 19.9±2.7**; pnd 21: 45.7±2.5, 43.4±6.9, 42.3±4.1*, 34.9±5.0** mean female pup bw (g): at birth: 5.8±0.4, 5.6±0.6, 5.4±0.3*, 3.2±0.1**; pnd 4: 8.5±1.0, 8.2±1.3, 8.2±1.1, 4.6±0.9**; pnd 7: 14.0±1.5, 13.4±2.4, 13.3±1.4, 7.0±1.5; pnd 14: 28.7±2.5, 25.9±3.5, 27.5±2.9, 17.1±4.4**; pnd 21: 43.9±3.3, 41.2±5.9, 39.8±3.8*, 30.3±6.0** pup viability (%): pnd 4: 93.7, 95.5, 97.7, 45.0**; pnd 21 male: 100, 92.0* 100, 82.4**/female: 98.0, 96.0, 100, 82.4**

post-mortem examinations:
pnd 4: no external abnormalities;
organ wt in males: absolute wt of lung (mg): 465±45, 442±48, 421±45*, 393±60*, of spleen (mg): 208±41, 177±45, 184±31, 141±50*, of liver (g): 1.95±0.15, 1.85±0.35, 1.82±0.26, 1.49±0.46*, of kidneys (g): 553±36, 526±91, 509±69, 423±130*, of testis (mg): 259±28, 227±52, 221±21**, 148±38**
relative wt of testis (mg/100 g bw): 561±49, 523±78, 519±42, 392±40**
organ wt in females: relative wt of heart (mg/100 g bw): 511±45, 541±56, 554±32* (no data for high dose)
pnd 21: no external/internal abnormalities; number of pups with skeletal abnormalities (%): 0/58, 0/55, 1/59 (2), 1/7 (15)**; number of pups with cervical ribs (%): 0/58 (0), 2/54 (4), 6/59* (10), 1/7 (15)**
pnd 49: organ wt in males: absolute wt of brain (g): 1.76±0.06, 1.73±0.06, 1.68±0.10*, 1.51±0.13**, of heart (mg): 958±96, 898±127, 917±128, 709±71**, of lung (g): 1.24±0.18, 1.16±0.11, 1.18±0.33, 0.84±0.03**, of spleen (mg): 635±90, 594±113, 580±108, 491±23**, of liver (g): 12.66±1.04, 11.63±1.88, 12.32±1.92, 9.23±0.36**, of kidneys (g): 2.18±0.14, 2.21±0.22, 2.12±0.36, 1.61±0.26**, of testis (g): 2.72±0.28, 2.72±0.42, 2.57±0.59, 1.85±0.26**
relative wt of brain (mg/100 g bw): 694±31, 733±63, 700±76, 858±11**, of heart (mg/100 g bw): 377±31, 378±23, 380±41, 403±3*
organ wt in females: absolute wt of brain (g): 1.66±0.07, 1.64±0.08, 1.58±0.10, 1.51±0.12*, of spleen (mg): 474±83, 478±85, 448±90, 385±1**, of kidneys (g): 1.54±0.12, 1.59±0.13, 1.52±0.16, 1.26±0.11*, of ovaries (mg): 63.2±9.5, 74.4±13.3*, 61.6±13.3, 60.0±9.9

					relative wt of ovaries (mg/100 mg bw): 35.6±4.5, 41.3±6.8*, 36.2±6.5, 41.3±0.8** postnatal development no effect on mean day of separation of auricle, emergence of abdominal hear, eruption of lower incisors, separation of eye lid, descent of testes, opening of vagina; at pnd 20: no effect on vision, hearing, pain sense, motility at pnd 28: open-field behaviour: mean ambulation/pup: 50.0±23.7, 62.8±24.9, 69.5±23.0, 72.8±32.0; mean rearing/pup: 7.7±4.5, 9.6±5.0, 12.1±6.4*, 11.0±3.5; mean grooming/pup: 1.0±1.6, 0.2±0.4*, 0.7±1.4, 0.6±0.9; no effect on preening, defecation, urination at pnd 35: no effect on rotarod performance at pnd 42: straight channel swimming test, mean time to reach goal (s): 77.8±21.6, 76.4±36.3, 60.2±20.4*, 55.5±11.9*; no effect on water T-maze test (performed 1 and 2 d later) at pnd 77: reproductive performance of F1: mean gestation period (d): 21.9±0.3, 21.2±0.8*, 20.9±1.1, 21.3±0.5* mean pup bw at birth: 6.5±0.3, 6.2±0.5, 6.1±0.3*, 6.1±0.4 reproductive performance (of F1 to produce F2): no effect on number of males mated, copulating, impregnating, % copulating/mating, % impregnation/mating, number of females mated, copulating, pregnant, % copulating/mating, % pregnant/mating number of implantations, mean implantations/litter, number live pups, mean live pups/litter, number of stillborns, stillborn rate, mean birth rate, sex ratio, number of pups with external abnormalities
Nagaoka/ Narama (1982)	female Sprague-Dawley rats (n=10/ group)	single administration on gd 7, 10 or 13	0, 5, 10 mg/kg bw; iv	no data on maternal toxicity	gd 7: total number of foetal deaths: 5, 11, 17* mean bw live foetuses (g): 3.55±0.17, 3.24±0.41*,

3.05±0.25**
mean body length live foetuses (cm): 3.7±0.07, 3.5±0.17*, 3.4±0.13**
mean tail length of liver foetuses (cm): 1.4±0.03, 1.3±0.10*, 1.3±0.07**
mean placental wt (g): 0.47±0.05, 0.43±0.05, 0.43±0.03*
gd 10:
total number of foetal deaths:4, 10, 55**
mean bw live foetuses (g): 3.56±0.22, 3.15±0.26**, 2.91±0.42**
mean body length live foetuses (cm): 3.6±0.08, 3.5±0.13, 3.3±0.24**
mean tail length of liver foetuses (cm): 1.4±0.05, 1.3±0.08**, 1.2±0.15**
mean placental wt (g): 0.43±0.05, 0.35±0.06**, 0.28±0.08*
number of foetuses with external abnormalities (%): 0, 13 (10)**, 62 (63)**; number of litters with abnormal foetuses: 0, 2, 6**; meningocele, encephalocele (%): 0, 12 (9)**, 54 (55)**; short/curly tail (%): 0, 1 (0.8), 11 (11)**, systematic oedema: 0, 0, 35 (367)**, cleft palate (%); 0, 0, 1 (1
number of foetuses with visceral abnormalities (%): 0, 2 (4), 20 (61)**; number of litters with abnormal foetuses: 0, 2, 6**; enlargement of subdural space with encephalon deformation (%): 0, 1 (2), 10 (30)**; deformation nasal cavities, olfactory bulb (%): 0, 0, 3 (9)*; deformation eye ball, retina, cornea (%): 0, 0, 15 (46)**; hydronephrosis defect, incomplete kidney development (%): 0, 0, 11 (33)**, subcutaneous oedema (%): 0, 0, 12 (36)**
number of foetuses with skeletal abnormalities (%): 0, 11 (13)**, 45 (69)**; number of litters with abnormal foetuses: 0, 3, 7**; rib abnormalities (%): 0, 10 (11)**, 39 (60)**, fusion, deformation of vertebrae (%): 0, 3 (3), 29 (45)**, partial cranium defect (%): 0, 9 (10)**, 38 (59)**

					gd 13: mean placental wt (g): 0.43±0.03, 0.42±0.04, 0.38±0.04** number of foetuses with external abnormalities (%): 0, 0, 35 (25)**; number of litters with abnormal foetuses: 0, 0, 3; meningocele, encephalocele (%): 0, 0, 35 (25)** number of foetuses with visceral abnormalities (%): 1 (1), 3 (7), 9 (20)**; number of litters with abnormal foetuses: 1, 2, 5**; enlargement of subdural space with encephalon deformation (%): 0, 0, 7 (15)** number of foetuses with skeletal abnormalities (%): 0, 11 (13)**, 26 (28)**; number of litters with abnormal foetuses: 0, 5**, 4*; rib abnormalities (%): 0, 10 (12)**, 7 (7)**, partial cranium defect: 0, 0, 21 (22)**
Nagaoka et al. (1982)	female Sprague-Dawley rats (n=20/group)	gd 17-pnd21; sacrifice dams: pnd 21; primary organ weighing; observationF1 for 77 d (function, motor coordination, behaviour, fecundity by mating to produce F2 generation, organ wt) and F2 (part of the pregnant F1 females sacrificed at gd 20; the remaining females allowed to litter; F2 pups sacrificed at weaning)	0, 2.5, 5, 10 mg/kg bw; iv	F0: no effect on bw; absolute wt of spleen (mg): 641±113, 511±76**, 460±69**, 438±83, of liver (g): 14.0±1.5, 13.1±1.9, 13.1±1.8, 12.7±1.7*; relative wt of spleen (mg/100 g bw): 212±30, 171±22**, 154±20**, 147±23**, of liver (g/100 g bw): 4.63±0.38, 4.39±0.43, 4.41±0.54, 4.24±0.34**	no effect on length of the gestational period, total number of implantations, parturition index, number of live pups, live litter size, percentage of viability for 4 d, the weaning rate; no external abnormalities number of stillborns: 0, 2, 6**, 11** mean male pup bw: at birth: 6.4±0.5, 6.3±0.4, 6.1±0.3*, 5.8±0.3**, at pnd 4: 9.3±1.2, 9.1±1.0, 8.7±0.8, 8.6±0.7*, at pnd 7: 14.5±1.6, 14.3±1.5, 13.8±0.9, 13.3±1.0**, at pnd 14: 28.2±2.9, 28.2±1.9, 26.7±1.5*, 25.9±2.1**, at pnd 21: 44.0±4.3, 44.2±3.2, 42.1±2.4, 39.1±3.3** mean female pup bw: at birth: 5.9±0.5, 6.0±0.3, 5.8±0.5, 5.5±0.4**, at pnd 4: 8.8±1.3, 8.9±1.0, 8.5±0.9, 8.1±0.8*, at pnd 7: 13.6±1.5, 14.1±1.0, 13.4±1.0, 12.6±1.1*, at pnd 14: 26.8±2.2, 27.8±1.7, 26.0±1.8, 24.8±1.4**, at pnd 21: 42.0±3.9, 43.2±2.5, 41.0±2.6, 37.6±2.2 pnd 21: male pup organ wt: absolute wt of liver (g): 1.77±0.29, 1.88±0.20, 1.78±0.16, 1.61±0.17*; relative wt of lung (mg/100 g bw): 1012±64, 992±56, 1011±82, 1109±127**, of liver (g/100 g bw):

4.14±0.26, 4.32±0.24*, 4.34±23*, 4.14±0.24, of kidney (mg/100 g bw): 1145±76, 1190±70, 1192±75, 1210±91*
female pup organ wt: absolute wt of heart (mg): 219±28, 223±22, 218±26, 201±19*, of liver (g): 1.77±0.26, 1.90±0.23, 1.82±0.22, 1.60±0.16*, of kidney (mg): 501±69, 533±60, 516±56, 463±48*, of ovary (mg): 114±27, 132±22*, 119±24, 111±23; relative wt of lung (mg/100 g bw): 1002±40, 1014±77, 1015±85, 1109±92**, of ovary (mg/100 g bw): 27.7±5.1, 31.0±4.6*, 29.1±5.2, 30.3±6.5
postnatal development
no effect on mean day of eruption of lower incisors, separation of eye lid, descent of testes, opening of vagina;
mean day of auricular separation: 2.1±0.2, 2.0±0.3, 2.4±0.4**, 2.5±0.4**, of emergence of abdominal hear: 0.9±0.5, 8.8±0.5, 8.6±0.6, 8.9±0.5
at pnd 20: no effect on vision, hearing, pain sense, motility
open-field behaviour: mean ambulation/pup: 47±18, 41±21, 52±18, 60±22**; mean rearing/ pup: 8.3±4.0, 7.4±5.3, 8.5±5.2, 11.5±6.4**; no effect on preening, grooming defecation, urination, on rotarod performance, on straight channel swimming test (1st day), water T-maze test (2nd day); water T-maze test 3rd day: time (s): 130±73, 139±61, 140±71, 257±401, error: 3.9±4.8, 5.5±6.2, 4.5±4.1, 24.5±55.1
at pnd 49: external examination: number of abnormal rats (%): 0, 0, 0, 39** (49), number of litters with abnormal rats: 0, 0, 0, 15**, hydrocephalic change: 0, 0, 0, 39; visceral examination: number of abnormal rats (%): 1 (3), 2 (5), 23 (62)**, number of litters with abnormal rats: 1, 2, 0, 16**, internal,external hydrocephalus (%): 0, 0, 0, 22 (60)**, unilateral hydronephrosis (%): 1 (3), 2 (5), 0, 1 (3); male organ wt: absolute wt of

heart (mg): 922±103, 922±85, 888±81, 825±82*, of lung (g): 1.20±0.15, 1.23±0.14, 1.15±0.13, 1.06±0.11**, of spleen (mg): 650±99, 685±97, 591±65*, 580±92*, of liver (g): 12.3±1.6, 12.3±1.4, 11.3±2.0, 10.3±2.0**, of kidney (g): 2.09±0.26, 2.08±0.22, 2.03±0.22, 1.79±0.19**, of testis (g): 2.60±0.23, 2.65±0.31, 2.61±0.25, 2.47±0.27**, relative wt of brain (mg/100 g bw): 713±66, 711±61, 757±62*, 798±86**, of kidney (mg/100 g bw: 837±79, 851±68, 889±63*, 823±47, of testis (mg/100 g bw): 1083±75, 1081±54, 1144±82*, 1137±102; female organ wt: absolute wt of heart (mg): 695±60, 711±79, 715±88, 638±75*, of lung (g): 0.96±0.11, 0.97±0.11, 0.97±0.08, 0.84±0.12**, of spleen (mg): 468±87, 479±85, 472±83, 393±96**, of liver (g): 8.5±1.0, 8.7±1.1, 8.6±1.1, 7.2±1.6**, of kidney (g): 1.48±0.13, 1.55±0.22, 1.48±0.14, 1.30±0.17**, relative wt of brain (mg/100 g bw): 956±71, 924±61, 953±61, 1175±321, of heart (mg/100 g bw): 395±35, 393±25, 411±36, 430±43**
reproductive performance (of F1 to produce F2):
no effect on % males copulating, impregnating, % females copulating, pregnant;
F1 pregnant females sacrificed at gd 20: no effect on mean number of corpora lutea/litter, mean number of implantations/ litter, implantation ratio, mean number of live foetuses/litter, foetal viability, mean foetal bw, number of external abnormalities; number of foetal deaths: 8, 6, 2, 11
F1 pregnant females allowed to litter: mean gestation period (d): 21.1±0.3, 21.3±0.4, 21.1±0.4, 20.8±0.5*; number of stillborns: 0, 1, 1, 3; no effect on mean litter size, viability on pnd 4, weaning rate, number of external abnormalities.

Nagaoka/ Narama (1982)	female Japanese white rabbits (n=5/ group)	gd 6-18; sacrifice: gd 29	0, 5, 10, 30, 50 mg/ kg bw/d; iv	50 mg kg bw: 100% mortality 30 mg kg bw: decreased bw	preliminary study 30 mg/kg bw: 100% foetal mortality 5, 10 mg/kg bw: 'mildly' increased foetal mortality (not quantified)
Nagaoka/ Narama (1982)	female Japanese white rabbits (n=10/ group)	gd 6-18; sacrifice: gd 29	0, 5, 10, 20 mg/kg bw/ d; iv	no effect on maternal bw, food and water consumption at 20 mg/kg bw: anaemia in 2/10 animals; at autopsy: organ wt: absolute wt of lung (g): 11.9±1.7, 13.1±1.6, 13.8±1.4*,12.2±1.7, of spleen (9): 1.38±0.51, 1.81±0.67, 1.90±0.44*, 2.09±0.74*, relative wt of lung (mg/ 100 g): 313±36, 352±47, 362±27**, 332±51, of spleen (mg/100 g bw): 35.6±10.4, 52.5±22.4, 50.0±12.4*, 57.0±21.5*, of kidney (mg/100g bw): 485±29, 548±77, 505±54, 514±44	main studies no effects on mean number of implantations/ litter, foetal deaths (early and late death, placental remnants, total), foetal deaths/litter, live foetuses, and live foetuses/ liter, on sex ratio, mean body length, mean placental weight, viability of live foetuses at 6 and 24 hr; mean number of corpora lutea/litter : 12.9±1.7, 11.2±2.4, 11.6±1.7, 10.1±0.9**; mean foetal bw (g): 37.1±5.4, 36.1±5.0, 33.1±3.1, 30.9±5.0* number of foetuses with external abnormalities (%): 0, 0, 1 (1), 7 (9); number of litters with foetuses with external abnormalities: 0, 0, 1, 4; ectrodactyla (%): 0, 0, 1, 7 (9)**; number of litters with skeletal abnormalities (%): 1 (1), 0, 1 (1), 22 (27)**, number of litters with foetuses with skeletal abnormalities: 1, 0, 1, 7**; vertebrae fusion: 0, 0, 0, 4*, rib deformation, shortening, defect: 0, 0, 0, 14**, sternebrae deformation, fusion, separation: 1, 0, 0, 5*, digitus defect: 0, 0, 1, 7**; number of foetuses with skeletal variations (%): 39 (40), 20 (25), 48 (49), 62 (76)**, limbar rib: 35, 18, 48, 58**, 7th sternebrae: 0, 3*, 0, 7**; no visceral abnormalities
Bus/Gibson (1973)	female Swiss Webster mice (n=6-7/group)	gd 11; sacrifice: gd 19	0, 5, 10, 20 mg/kg bw; ip	not reported	gross abnormalities (%): open eyes: 0, 0, 7.8±5.0, 90.0±10.0*; external hydrocephalus, 0, 1.3±1.3, 1.3±1.3, 90.0±90.0*; micromelia: 0, 3.9±3.9, 0, 63.3±18.6*; adactyly: 0, 0, 15.8±13.9, 83.3±16.7*; syndactyly: 0, 1.0±1.0, 2.4±2.4, 65.0±15.9*; microcaudate: 0, 0, 0, 78.3±14.8*; kinked tail: 0, 1.0±1.0, 1.3±1.3, 26.7±19.4 soft tissue abnormalities (%): cleft palate: 0, 0, 9.3±4.4,

		20.0±20.0; internal hydrocephalus: 0, 0, 9.9±4.9, 90.0±10.0*; microphakia: 0, 0, 7.1±7.1, 46.7±22.0*; kidney ectopia: 0, 0, 2.4±2.4, 66.7±21.1*; hydronephrosis: 0, 0, 0, 76.7±14.5*; exencephaly: 0, 0, 0, 10.0v10.0; cryptorchidism: 0, 0, 20.8±16.3, 40.0±24.5 skeletal abnormalities (%): absent, not ossified bones: 0, 0, 0, 66.7±33.3*; absent, not ossified vertebrae: 0, 4.8±4.8, 6.5±4.4, 55.6±29.4*; fused sternebrae: 0, 3.3±3.3, 22.0±10.8, 44.4±29.4*; supernumerary ribs: 0, 55.2±14.1*, 10.4±8.2, 0; fused ribs: 0, 0, 0, 100*; absent, not ossified ribs: 0, 0, 2.1±2.1, 16.7±16.7; fused vertebrae: 0, 0, 49.8±16.1*, 100*; absent, not ossified fibula: 0, 0, 0, 100*; absent, not ossified ulna: 0, 0, 0, 61.1±20.0*; absent, not ossified radius: 0, 0, 0, 61.1±20.0*; absent, not ossified metatarsals: 0, 2.4±2.4, 25.3±13.0, 61.1±20.0*; absent, not ossified metacarpals: 0, 0, 25.3±13.0, 77.8±22.2*; absent, not ossified phalanges: 0, 15.2±10.5, 32.1±15.1, 61.1±20.0*; exostosis: 0, 0, 4.2±4.2, 16.7±16.7

abbreviations: bw=body weight; d=day(s); gd=gestational day(s); hr=hour(s); ip=intraperitoneal; iv=intravenous; n=number(s); pnd=postnatal day(s); s=second(s); wt=weight(s).
*: $p<0.05$; **: $p<0.01$

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

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