Isopropyl methanesulphonate

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies 'Isopropyl methanesulphonate'
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Mijnheer de staatssecretaris,

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

In dit kader bied ik u hierbij een advies aan over isopropyl methaansulfonaat. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb deze publicatie heden ter kennisname aan de Minister van Volksgezondheid, Welzijn en Sport en aan de Staatssecretaris van de Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

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Evaluation of the effects on reproduction, recommendation for classification

Committee for Compounds Toxic to Reproduction,
a committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

No. 2004/07OSH, The Hague, September 9, 2004

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad isopropyl methaansulfonaat (IPMS) onder de loep genomen. IPMS is een direct alkylerende verbinding, die met name gebruikt wordt als modelstof in biomedisch onderzoek. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de raad, kijkt naar effecten op de vruchtbaarheid van mannen en vrouwen zowel als op de ontwikkeling van het nageslacht. Bovendien worden effecten van blootstelling van de zuigeling via de moedermelk beoordeeld.

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

- Voor effecten op de fertiliteit adviseert de commissie om IPMS te classificeren in categorie 3 (*Stoffen die in verband met hun mogelijke voor de vruchtbaarheid van de mens schadelijke effecten reden geven tot bezorgdheid)* en te kenmerken met Xn;R62.
- Voor effecten op de ontwikkeling, adviseert de commissie om IPMS te classificeren in categorie 3 (*Stoffen die in verband met hun mogelijke voor de ontwikkeling van de mens schadelijke effecten reden geven tot bezorgdheid)* en te kenmerken met Xn;R63.

- Voor effecten tijdens lactatie, adviseert de commissie om IPMS niet te kenmerken wegens onvoldoende geschikte gegevens.

Op grond van wat bekend is uit onderzoek naar effecten op de fertiliteit en de ontwikkeling als gevolg van blootstelling aan IPMS, classificeert de commissie IPMS in categorie 3 (er is reden voor bezorgdheid). Op basis van het toxicologisch werkingsmechanisme van direct alkylerende stoffen kan echter gesteld worden dat IPMS waarschijnlijk een reproductietoxische stof is.

Executive summary

In the present report the Health Council of the Netherlands reviewed isopropyl methanesulphonate (IPMS). IPMS is a direct alkylating substance, which is among other things used as a model compound in biomedical research. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Committee for Compounds toxic to reproduction, a committee of the Health Council, evaluates the effects on male and female fertility and on the development of the progeny. Moreover the effects of exposure on lactation are considered.

According to the Directive 93/21/EEC of the European Union, the committee recommends a classification. The committee's recommendations for IPMS are:

- For effects on fertility, the committee recommends classifying IPMS in category 3 (*Substances which cause concern for human fertility)* and labelling IPMS with Xn; R62.
- For developmental toxicity, the committee recommends classifying IPMS in category 3 (*Substances which cause concern for humans owing to possible developmental toxic effects)* and labelling IPMS with Xn; R63.
- For effects during lactation, the committee is of the opinion that IPMS should not be labelled due to a lack of appropriate data.

Based on the available studies concerning the effects of exposure to IPMS on fertility and development, the committee recommends classifying IPMS in category 3 (cause concern for humans). However, general information about the mechanism of toxicity of direct alkylating compounds shows that adverse effects on reproduction are to be expected after exposure to IPMS.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Health Council's Committee for Compounds toxic to reproduction according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (category 1, 2 or 3) or labelled as may cause harm to breastfed babies (R64).

1.2 Committee and procedure

The present document contains the classification of isopropyl methanesulphonate by the Health Council's Committee for Compounds toxic to reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by Ing. JJA Muller at the Centre for Substances and Integrated Risk Assessment of the RIVM Bilthoven, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development and lactation of the above mentioned compound.

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

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

In 2003, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

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

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective of the general toxic effects (see Annex C, 4.2.3.1 category 1).
- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.
- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur at a higher incidence than in the control group in the absence of maternal

effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).

- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterisation and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceedence of the exposure limit for the general population, eg the acceptable daily intake (ADI).

1.5 Data

Literature searches were conducted in the on-line databases Toxline starting from 1985 up to June 2001, Toxcenter from 2000 up to January 2003 and Medline starting from 1966 up to January 2003. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection

* Organisation for Economic Cooperation and Development

of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited.

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

1.6 Presentation of conclusions

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

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

1.7 Final remark

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

* for definitions see Tox95

Chapter 2

Isopropyl methanesulphonate (IPMS)

2.1 Introduction

Name	:	Isopropyl methanesulphonate
CAS-no	:	926-06-7
Synonyms	:	2-Propyl methanesulphonate, methanesulfonic acid, 1-methylethyl ester, IPMS, IMS
Use	:	Alkylating agent, biomedical research, experimental mutagen.
Mol weight	:	138.18
Chem formula	:	$C_4H_{10}O_3S$
Conversion factor	:	1 ppm = 5,65 mg/m^3 (101 kPa, 25°C) 1 mg/m^3 = 0.177 ppm 1% = 56500 mg/m^3

Isopropyl methanesulphonate (IPMS) is a genotoxic carcinogen (can react with nucleic acids) and directly reacts with the cellular DNA (direct alkylating). It has been and still is used for the study of toxic events, resulting from the alkylation of tissue macromolecules, notably DNA. Depending on the ability of the compound to reach the molecular target, direct alkylating compounds may also react with the genetic material of germ cells and somatic cells of the foetus. As mutations can be the basis of abnormal development, the Committee Compounds toxic to reprotduction is of the opinion that due to these properties, effects on reproduction may be expected after exposure to IPMS.

2.2 Human studies

Fertility

There are no studies available.

Development

There are no studies available.

Lactation

There are no studies available.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarize the fertility and developmental toxicity studies with IPMS in experimental animals.

General introduction

IPMS is an alkylating substance. Studies have been performed to acquire insight into the heritable effects of IPMS, either via a direct action on the fertilised egg, embryo and foetus or on the effects of these substances on gametogenesis and gametes in males.

However, for IPMS standard fertility or developmental toxicity studies are not available. Most of the studies are limited to parenteral (in particular intraperitoneal) with usually single dosing. Information on general or parental toxicity is commonly not provided, but in some studies, parental lethality has been observed. In other studies, not necessarily at lower dose levels, no effects on parental survival or even sublethal effects have been mentioned.

In the subsequent sections, a number of studies have been reviewed in more detail. These are chosen because they provide a sufficient level of experimental detail, or because they are illustrative for the effects of IPMS. By no means are these studies performed according to regulatory guidelines for reproductive toxicity studies.

Fertility studies

No standard fertility studies with IPMS are available (see table 1.1, Annex D).

IPMS reduced the number of spermatogonia in the testicular tubules of male rats resulting in a complete depletion of spermatogonia, spermatocytes, spermatids and spermatozoa on day 30 after a single i.p. injection with 50 or 100 mg/kg IPMS (Jac61). Fertility was reduced from week 3 until week 15.

In male mice, a comparable effect was seen (Roo70). Treatment with a single i.p. injection with 25 mg/kg or above, resulted in the aplasia of all A and I spermatogonia as concluded from the complete depletion up to the pachytene spermatocytes in stage 9 after 12 days.

In other studies in rats, mice and rabbits, comparable effects on male fertility were found after parenteral single dosing (Fox63, Par63, Par64, Bis97, Lie97).

Several dominant lethal studies are available in which effects were found in male and female animals after i.p. injection with IPMS (see table 1.2, Annex D).

Male mice received a single i.p. injection with 0, 20, 40, 50, 80 or 100 mg/kg bw IPMS and were mated with one female per male for every 4 days up to day 48 (Ehl95). Females were sacrificed on day 14-17 after mating. The male mice injected with 20 mg/kg bw showed a dose dependent decrease in fertility (induced dominant lethals) from day 25. Complete sterility was observed from day 33 after treatment with 80 mg/kg and from day 37 after treatment with 40 mg/kg. The percentage of pre-implantation loss was dose-dependently increased especially in the mating intervals preceding the infertile periods. Post-implantation loss was only increased at the two highest dose levels.

Treatment of female mice with a single i.p. injection with 50 or 75 mg/kg resulted in a reduction of the time to first birth (Gen71b). After the first interval, the number of productive females was normal but the litter size was reduced up to day 82. After day 157, the litter size remained below those in controls. Histological examination of the ovaries on day 3 and 102 after treatment revealed a decrease in oocytes of 50% and 90%, respectively.

Developmental toxicity

In Annex D, table 2.1, tests are included which have specifically paid attention to morphological changes in the offspring of dams exposed to IPMS. Embryo- and foetal lethality are covered by the dominant lethal studies in Annex D (see table 1.2). However, it should be noted that in some *in vivo* developmental studies embryo- or foetotoxicity resulting in increased pre- or post-implantation loss was also observed.

No standard developmental studies with IPMS are available. There are only two studies in which pregnant rats were treated.

Hemsworth (Hem68), treated groups of 5 pregnant dames on day 13 post coitum with a single i.p. injection (0, 50 or 75 mg/kg of IPMS). The dams were killed on day 20 post-coitum and the foetuses were examined for gross anatomical deformities. The highest dose (75 mg/kg) induced a small increase in non-viable implants (4 in 5 litters versus 1 in 5 litters in controls) and a strong increase in foetuses with syndactyly (21/39 versus 0/36). At 50 mg/kg bw, 2 out of 40 foetuses showed limb defects.

Furthermore, groups of pregnant rats (n=4 per group) were treated on day 5, 9, 12, 13, 14, 15 or 16 of gestation with 50 mg/kg bw or on day 16 with 75 or 100 mg/kg bw. The number of offspring was determined. Treatment with 50 mg/kg on day 14 or later resulted in near normal numbers of offspring. Earlier treatment resulted in a strong decrease in the number of offspring. Higher doses (75 and 100 mg/kg) on day 16 resulted in a dose dependent reduction in the number of offspring (2.7 and 0 versus approximately 7 in controls).

Moreover, five consecutive daily i.p. injections (10 mg/kg bw) on day 12 to 16 did not affect the litter size.

Treatment of dams on day 16 post coitum with a single i.p. injection (50 mg/kg bw IPMS) caused a small increase in testicular tubules without germ cells on day 1 and 6 months post partum and a strong increase (86%) on day 15 post partum.

Treatment of pregnant dams with 50 mg/kg on day 16 or 20 and treatment of neonates on day 2, 4 and 7 post partum induced a strong reduction of the number of type A spermatogonia in the testicular tubules of the male neonates on day 15 post partum. Treatment of pregnant dams with 50 mg/kg on day 13, 14, 15, 16 or 20 did not affect the fertility of the male offspring when they were at least 4 months old. Treatment of pregnant dams with 50 mg/kg on day 15, induced a small reduction (21%) of the testis weight in 6 month old male offspring. No information is provided on the maternal effects of the treatment.

In another study of Hemsworth (Hem69), female rats were treated with a single i.p. injection on day 13, 14, 15, 16 or 20 of gestation. Offspring of three litters were killed on days 1 and 15 post partum and their ovaries were examined. The remaining litters were kept to maturity (6 months or 1 year) for assessment of their fertility.

Injection on day 16 had a strong destructive action on oogonia and resulted in elimination of germ cells from offspring. Ovaries from dams treated on day 20 appeared normal. Treatment of the dams on day 13, 14, 15 or 16, resulted in a strong decrease of fertility of the offspring as shown by the incapability to become pregnant. Treatment on day 20 did not affect the fertility of the offspring. The ovary weight of the offspring of

females treated on day 16 of gestation was reduced with 69% at 6 months after birth. No information is provided on the maternal effects of the treatment.

Lactation

No studies on IPMS in breast milk were available.

2.4 Conclusion

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

Several animal studies show that IPMS affects fertility in male and female animals. IPMS has a short term effect by inducing mutations in spermatozoa and ova which result in pre- and post-implantation loss. Furthermore, IPMS has a cytotoxic effect on spermatogonia resulting in reduced fertility after a prolonged period. In females, a long term effect on fertility was observed via the reduction of ova numbers. These effects are clear evidence of a reduction of the fertility with supporting evidence on mechanism of action and site of action. However, all these effects were seen after i.p. administration. The relevance for humans is doubtful because the route of administration is inappropriate. Furthermore, several authors state that IPMS is unstable in aqueous solution at body temperature. Jackson *et al* (1961) reported a half-life of 13 minutes at 37°C in aqueous solution. Only one study compared oral versus i.p. administration of IPMS in laboratory animals (Adl96) and found comparable numbers of micronuclei in the bone marrow of mice after i.p. and p.o. administration, suggesting that the systemic availability may be comparable. Therefore, the committee recommends classifying in category 3 ('substances which cause concern for human fertility') and labelling IPMS with R62 ('possible risk of impaired fertility'). However, general information about the mechanism of toxicity of direct alkylating compounds shows that adverse effects on fertility are to be expected after exposure to IPMS.

No studies on the effect of IPMS on human development were available. Only two studies on the effect on development in laboratory animals were available. The studies showed that IPMS induces embryotoxic/foetotoxic effects, structural defects and organ toxicity (testis and ovaries) after a single i.p. injection. No information is provided on the maternal effects of the treatments. However, the relevance for humans is doubtful because the route of administration is inappropriate. But again, a study by Adler *et al* (Adl96) suggests that the systemic availability after i.p and po administration may be comparable. Therefore, the committee recommends classifying IPMS in category 3 ('substances which cause concern for humans owing to possible developmental toxic effects') and to label with R63 ('may cause harm to the unborn child'). However, gen-

eral information about the mechanism of toxicity of direct alkylating compounds shows that adverse effects on development are to be expected after exposure to IPMS.

As no studies on IPMS in breast milk were available, the committee recommends that IPMS should not be labelled for effects during lactation because of a lack of appropriate data.

Proposed classification for fertility

Category 3, Xn;R62

Proposed classification for developmental toxicity

Category 3, Xn;R63

Proposed labelling for effect during lactation

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

References

Adl96 Adler ID, Tinwell H, Kliesch U, Ashby J. Activity of iPMS and nPMS in mouse bone marrow micronucleus assays: comparison with mouse dominant lethal assay data. Mutation Research 1996; 349: 241-247.

Bis83 Bishop JB, Kodell RL, Whorton EB, Domon OE. Dominant lethal test response with IMS and TEM using different combinations of male and female stocks of mice. Mutation Research 1983; 121: 273-80.

Bis97 Bishop JB, Morris RW, Seely JC, Hughes LA, Cain KT, Generoso WM. Alterations in the reproductive patterns of female mice exposed to xenobiotics. Fundam. Appl. Toxicol. 1997; 40: 191-204.

Ehl72 Ehling UH, Doherty DG, Malling HV. Differential spermatogenic response of mice to the induction of dominant-lethal mutations by n-propyl methanesulphonate and isopropyl methanesulphonate. Mutation Research 1972; 15: 175-184.

Ehl95 Ehling UH, Neuhauser-Klaus A. Induction of specific-locus and dominant lethal mutations in male mice by n-propyl and isopropyl methanesulphonate. Mutation Research 1995; 328: 73-82.

Fox63 Fox BW, Jackson H, Craig AW, Glover TD. Effects of alkylating agents on spermatogenesis in the rabbit. J. Rprod. Fertil. 1963; 5: 13-22.

Gen71a Generoso WM, Huff SW, Stout SK. Chemically induced dominant-lethal mutations and cell killing in mouse oocytes in the advanced stages of follicular development. Mutation Research 1971; 11: 411-420.

Gen71b Generoso WM, Stout SK, Huff SW. Effects of alkylating chemicals on reproductive capacity of adult female mice. Mutation Research 1971; 13: 171-184.

Gen79a Generoso WM, Huff SW, Cain KT. Relative rates at which dominant-lethal mutations and heritable translocations are induced by alkylating chemicals in postmeiotic male germ cells of mice. Genetics 1979; 93: 163-171.

Gen79b Generose WM, Cain KT, Krishna M, Huff SW. Genetic lesions induced by chemicals in spermatozoa and spermatids of mice are repaired in the egg. Proc. Natl. Acad. Sci. 1979; 76: 435-437.

Hem68 Hemsworth BN. Embryopathies in the rat due to alkane sulphonates. J. Reprod. Fert. 1968; 17: 325-334.

Hem69 Hemsworth BN. Effect of alkane sulphonic esters on ovarian development and function in the rat. J. Reprod. Fert. 1969; 18: 15-20.

Jac61 Jackson H, Fox BW, Craig AW. Antifertility substances and their assessment in the male rodent. J. Reprod. Fertil. 1961; 2: 447-465.

Kat83 Katoh M, Iwahara S. Relationship between chromosome aberrations at the first cleavage metaphase and postimplantation loss in dominant lethal mutations induced by isopropyl methanesulphonate. Jpn. J. Genet. 1983; 58: 345-351.

Kra78 Kratochvilova J. Evaluation of pre-implantation loss in dominant-lethal assay in the mouse. Mutation research 1978; 54: 47-54.

Lie97 Liegibel UM, Schmezer P. Detection of the two germ cell mutagens ENU and iPMS using the LacZ/ transgenic mouse mutation assay. Mutation Research 1997; 388: 213-218.

Mat97a Mattison JD, Penrose LB, Burlinson B. Preliminary results of ethylnitrosourea, isopropyl methanesulphonate and methyl methanesulphonate activity in the testis and epididymal spermatozoa of Muta Mice. Mutation Research 1997; 388: 123-127.

Par63 Partington M, Jackson H. The induction of dominant lethal mutations in rats by alkane sulphonic esters. Genet. Res. 1963; 4: 333-345.

Par64 Partington M, Fox BW, Jackson H. Comparative action of some methane sulphonic esters on the cell population of the rat testis. Experimental Cell Research 1964; 33: 78-88.

Roo70 Rooij DG de, Kramer MF. The effect of three alkylating agents on the seminiferous epithelium of rodents. Virchows Arch. Abt. B Zellpath. 1970; 4: 267-275.

Sut76 Suter KE, Generoso WM. Chemical induction of presumed dominant-lethal mutations in postcopulation germ cells and zygotes of mice. II. Sensitivity of different postcopulation-precleavage stages to three alkylating chemicals. Mutation Research 1976; 34: 259-270.

Tox95 Niesink RJM, de Vries J, Hollinger MA, eds, Toxicology, Principles and Applications, Boca Raton: CRC Press, 1995: 385.

Tin97 Tinwell H, Lefevre P, Williams CV, Ashby J. The activity of ENU, iPMS and MMS in male mouse germ cells using the Muta Mouse positive selection transgenic mutation assay. Mutation Research 1997; 388: 179-185.

Literature consulted but not cited

Ash95 Ashby J. Series: current issues in mutagenesis and carcinogenesis, No. 54. Rodent germ cell mutagens and their activity in the rodent bone marrow micronucleus assay. Mutation Research 1995; 328: 239-241.

Ash97 Ashby J, Gorelick NJ, Shelby MD. Mutation assays in male germ cells from transgenic mice: Overview of study and conclusions. Mutation Research 1997; 388: 111-122.

Dou97 Douglas GR, Gingerich JD, Soper LM, Jiao J. Toward an understanding of the use of transgenic mice for the detection of gene mutations in germ cells. Mutation Research 1997; 388: 197-212.

Eps72 Epstein SS, Arnold E, Andrea J, Bass W, Bishop Y. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicology and applied Pharmacology 1972; 23: 288-325.

Gen80 Generoso WM. Repair in fertilized eggs of mice and its role in the production of chromosomal aberrations. Basic Life Sci. 1980; 15: 411-420.

Gor97 Gorelick NJ, Andrews JL, Gibson DP, Carr GJ, Aardema MJ. Evaluation of lacI mutation in germ cells and micronuclei in peripheral blood after treatment of male lacI transgenic mice with ethylnitrosourea, isopropylmethane sulphonate or methylmethane sulphonate. Mutation Research 1997; 388: 187-195.

Jel89 Jelovsek FR, Mattison DR, Chen JJ. Prediction of risk for human developmental toxicity: How important are animal studies for hazard identification? Obstet. Gynecol. 1989; 74: 624-636.

Kat94 Katoh M, Inomata T, Horiya N, Suzuki F, Shida T, Ishioka K, Shibuya T. Studies on mutations in male germ cells of transgenic mice following exposure to isopropyl methanesulphonate, ethylnitrosourea or X-ray. Mutation Research 1994; 341: 17-28.

Kra70 Kramer MF, de Rooij DG. The effect of three alkylating agents on the seminiferous epithelium of rodents. Virchows Arch. Abt. B Zellpath. 1970; 4: 276-282.

Mat97a Mattison JD, Penrose LB, Burlinson B. Preliminary results of ethylnitrosourea, isopropyl methanesulphonate and methyl methanesulphonate activity in the testis and epididymal spermatozoa of Muta Mice. Mutation Research 1997; 388: 123-127.

Mat97b Mattison JD, Penrose LB, Burlinson B. Preliminary results of ethylnitrosourea, isopropyl methanesulphonate and methyl methanesulphonate activity in the testis and epididymal spermatozoa of Muta mice. Mutagenesis 1997; 12: 104.

Ols93 Olshan AF, Faustman EM. Male-mediated developmental toxicity. Annu. Rev. Public Health 1993; 14: 159-181.

Pie97 Piegorsch WW, Lockhart AC, Carr GJ, Margolin BH, Brooks T, Douglas GR, Liegibel UM, Suzuki T, Thybaud V, van Delft JH, Gorelick NJ. Sources of variability in data from a positive selection lacZ transgenic mouse mutation assay: An interlaboratory study. Mutation Research 1997; 388: 249-289.

Pro97 Provost GS, Rogers BJ, Dycaico MJ, Carr G. Evaluation of the transgenic Lambda/LacI mouse model as a short-term predictor of heritable risk. Mutation Research 1997; 388: 129-136.

Put97 Putman DL, Ritter AP, Carr GJ, Young RR. Evaluation of spontaneous and chemical-induced lacI mutations in germ cells from lambda/lacI transgenic mice. Mutation Research 1997; 388: 137-143.

Sch85 Schardein L, Schwetz BA, Kenek MF. Species sensitivities and prediction of teratogenic potential. Environmental Health Perspectives 1985; 61: 55-67.

Sch93 Schardein JL. Chemically Induced Birth Defects. 1993; 842-869.

She97 Shelby MD, Tindall KR. Mammalian germ cell mutagenicity of ENU, IPMS and MMS, chemicals selected for a transgenic mouse collaborative study. Mutation Research 1997; 388: 99-109.

Smi83 Smith MK, Kimmel GL, Kochhar DM, Shepard TH, Spielberg SP, Wilson JG. A selection of candidate compounds for in vitro teratogenesis test validation. Teratogenesis, Carcinogenesis and Mutagenesis 1983; 3: 461-480.

A The Committee

B Comments on the public draft

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

D Fertility and developmental toxicity studies

E Abbreviations

Annexes

Annex **A**

The committee

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The first draft of the present document was prepared by Ing. JJA Muller from the RIVM in Bilthoven, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance:
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Annex **B**

Comments on the public draft

A draft of the present report was released in 2003 for public review. The following persons and organisations have commented on the draft document:

- RD Zumwalde,
  Centers for Disease Control and Prevention, NIOSH, USA

Annex **C**

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

4.2.3 Substances toxic to reproduction

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

Category 1:

Substances known to impair fertility in humans

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

Substances known to cause developmental toxicity in humans

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

Category 2:

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

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

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

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

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

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

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

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

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

Generally on the basis of:

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

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

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

T; R60: May impair fertility

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

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

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

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

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

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

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

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

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

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

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

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

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

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

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

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

Developmental toxicity

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

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

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

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

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

Annex **D**

Fertility and developmental toxicity studies

Table 1.1 Fertility studies in animals.

authors	species	experimental period/design	dose and route	general toxicity	Effects on reproductive organs and reproduction
Jac61	Male rats	Treated rats were mated with females at weekly intervals.	Rats received a single i.p. injection with 50 or 100 mg/kg bw	Weight loss following a large single dose but not following small divided doses	Fertility was reduced from week 3 until week 15. Both doses induced a block in the formation of spermatocytes from their predecessors resulting in a complete depletion from day 30.
Fox63	Male rabbits of Dutch and English breed	Ejaculates were collected weekly or twice a week for up to 12 weeks after treatment.	Single intravenous injection with 100 mg/kg bw	Not stated	Increase in spermatozoa with abnormalities from day 45 upto week 12. The number of spermatozoa per ejaculate began to fall in week 7 with a minimum value at week 11 and being followed by a rapid recovery.
Par63	Male rats (n=8)	Males were mated with one female from week 5 to 8 after treatment. Ova were examined at 36 to 40 hours post coitum	Single i.p. injection with 0 or 50 mg/kg bw	Not stated	An increase in abnormal fertilized ova was seen in week 5.
Par63	Male rats (n=2)	Spermatid numbers per tubule were determined at various days after treatment.	Single i.p. injection with 0 or 50 mg/kg bw	Not stated	Spermatid numbers were strongly reduced on day 26 and zero on day 30.
Par64	Male rats (n=2)	Rats were sacrificed on several times after treatment up to 20 days. The testes were removed and the number of seminiferous epithelium cells in spermatogenic stages was determined.	Single i.p. injection with 0, 50 or 100 mg/kg bw.	Not stated	Strong reduction in spermatogonia type A within 1 or 2 days at both doses and total disappearance of later stages at later timepoints. At the lowest dose there were some indications of recovery. The primary effect was on the later stages of Type A development (stages XII to XIV). At the higher dose, stages I to IV of Type A development were also affected.

Roo70	Male mice of strain N (n=2 per dose and period)	Mice were killed on different days after treatment and the depletion of the seminiferous epithelium of the testis was examined.	Mice received a single i.p. injection with 10, 25, 50, 100 or 200 mg/kg bw.	Not stated	Treatment with 25 mg/kg or higher killed all A and I spermatogonia after 12 days. A dose of 200 mg/kg killed all spermatogonia and part of the primary spermatocytes.
Lie97	Male LacZ transgenic CD2F1 mice (n=7-8)	Animals were sacrificed on day 52	Single i.p. injection with 100 mg/kg bw	Slightly reduced body weight gain	Testes weights were 27% of controls.
Bis97	Mice of different strains (n=30 or 36)	Females were caged with one male and the total reproductive performance during 347 days was determined	Single i.p. injection of the females with 0, 50 or 75 mg/kg bw.	Not stated	Significant reduction in number of litters and number of offspring per female

Table 1.2 Dominant lethal tests in animals.

authors	species	experimental period/design	dose and route	general toxicity	Effects on reproductive organs and reproduction
Par63	Rats (n=6)	Treated male rats were mated with one female per week during 10 weeks. Females were sacrificed on day 14 after mating.	Male rats received a single i.p. injection with 0 or 50 mg/kg bw	Not stated	Pre-implantation loss was increased from week 2 to week 10. Post-implantation loss was increased in week 7. Percentage of live implants was reduced from week 4 to week 10. No live implants were seen in week 9
Ehl72	Male mice (101 x C3H)F1 and female mice ((101 x C3H)F1 or C3H x 101)F1). Twenty females per interval.	Treated male mice were mated with one female mouse for periods of 2 or 4 days up to day 46. Pregnant females were killed on day 14-17 after mating.	Male mice received a single i.p. injection with 0, 50, 100 or 200 mg/kg bw.	Highest nonlethal dose was 200 mg/kg	The fertility was dose-dependently decreased. At the lower doses, the fertility was decreased in males mated before day 7 and after day 23. All mating intervals were affected at 200 mg/kg. The number of implants and live embryos per female were dose-dependently decreased, especially at the early and late intervals.
Sut76	Mice (C3H x 101)F1	Females were sacrificed on day 12-14 after treatment.	Single i.p. injection with 0 or 25 mg/kg bw at several timepoints between 4.5 and 17.5 hours after mating.	Not stated	The percentage of dead implants was increased above control levels at all timepoints and increased from 14% at 4.5 hours after mating to 40% at 17.5 hours.
Kra78	Mice (101 x C3H)F1 (n=40)	After injection each male was caged with one female for 2 (first period) or 4 days until day 38. Females were sacrificed on day 14-17 after mating.	Single i.p. injection of males with 50 mg/kg bw	Not stated.	Increase of post-implantation loss was seen in females mated on day 1 to 14 and an increase in unfertilized ova from day 27.
Gen79b	Male mice (101 x C3H)F1 or (C3H x 101)F1 and 4 different stocks of female mice. Number per group not stated.	Males treated were mated with two females from day 0.5 to 3.5 (65 mg/kg) or day 3.5 to 7.5 (125 mg/kg) post treatment. Females were sacrificed on day 12-15 after mating.	Single i.p. injection of male mice with 65 or 125 mg/kg bw	Not stated	Treatment with 125 mg/kg induced a reduction of pregnancies. Both treatments induced a reduction in living embryos per female (variations depending on female strain).

Kat83	Mice (C57Bl/6 x DBA/2) (n=8 to 23 per mating period)	Males were mated with one female per week up to 5 weeks after treatment. Females were killed on day 13 after mating.	Male mice received a single i.p. injection with 0 or 200 mg/kg bw.	Not stated	Treatment induced a reduction of pre- and post-implantation loss at all mating periods. From day 27, no implants were found in females with vaginal plug.
Kat83	Mice (C57Bl/6 x DBA/2) (n=14)	Males were mated with one female on day 7 to 10 after treatment. Females were killed 72 hour after ovulation.	Male mice received a single i.p. injection with 0 or 200 mg/kg bw.	Not stated	IPMS induced a strong reduction in the number of cleavage stages in the embryos.
Bis83	Male mice (n=16): 3 different strains. Female (n=48): 2 different strains.	Caging of 1 male with 3 females of all strain combinations on day 0.5 to day 4. Females were sacrificed on day 13 after the mating interval.	i.p.: 0.65 mg/kg bw on day 1 (males only)	Not stated	Increased fetal death rate with all strain combinations compared to controls.
Ehl95	Mice (102/El x C3H/El)F1 (n=25, 50 or 60)	After injection each male was caged with one female for 4 days up to day 48. Females were sacrificed on day 14-17 after mating. Number of corpora lutea, dead and total implants were determined	Single i.p. injection of the males with 0, 20, 40, 50, 80 or 100 mg/kg bw	Not stated	Dose-dependent decrease in fertile matings, copora lutea per female, implants per female and live embryos per female from 20 mg/kg at day 25 and later. Sterility from 40 (respectively 80) mg/kg bw at day 37 (respectively 33) and beyond. Increase in dead implants from 80 mg/kg bw.
Tin97	Muta Mouse (n=6)	Male mice were mated with two females on day 10. Females were sacrificed on day 17 post coitum. Male mice were mated with two females on day 40 and pre-dosing to determine the number and cell number of ova's on day 2 post coitum. Caudal sperm counts on day 50.	Single i.p. injection of the males with 0 or 100 mg/kg bw	Not stated	An increase of early death embryos was seen after mating with males treated 10 days before. Only ova consisting of one cell were found in females after mating with males treated 40 days before. This indicates no fertilization. Sperm counts on day 50 were low or zero.
Gen71a	Three strains of female mice (n=29 to 42 per mating interval and strain) and one strain of male mice	Females were mated with untreated males on day 0.5-4.5, 5.5-9.5, 10.5-14.5 or 15.5-19.5 post treatment. Females were sacrificed on day 12-15 after mating.	Single i.p. injection of females with 75 mg/kg bw	Stated to be the highest dose without death or weight loss	Strain dependent reduction of fertility, total implants and living embryos among fertile females especially in the early mating intervals. Ovulation was reduced in females mated within 4.5 days after treatment. Almost no normal follicles were seen on day 2 or 3 after treatment.
Gen71b	Female mice (SEC x C57Bl)F1 (n=30 or 59) and male mice (101 x C3H)F1	Treated female mice were continuously caged with one male mouse. All newly born mice were scored and removed. Oocytes were counted on day 3 or 102 after treatment (n=2).	Female mice received a single i.p. injection with 0, 50 or 75 mg/kg bw.	Not stated	Dose-dependent reduction of the time to first birth and numbers of nest and young per female. The number of oocytes per female was reduced to respectively 50% and 10% of control values at day 3 and 103 after treatment.
Gen79a	Male (SEC x C57Bl)F1 and female (C3H x C57Bl)F1 mice (n= between 17 and 52 females per group)	Females were treated just before or just after mating. Females were sacrificed on day 12-15 after mating.	Female mice received a single i.p. injection with 50 mg/kg bw.	Not stated	Treatment before mating resulting in 50% dead implants and after treatment in 25%. This indicates that sperm and eggs have the same sensitivity for IPMS.

Table 2.1 Developmental studies in animals with isopropyl methanesulphonate.

authors	species	experimental period/ design	dose and route	general toxicity	Effects on reproductive organs and reproduction
Hem68	Wistar rats (n=5/group)	Treatment on day 13 post coitum. Dams were sacrificed on day 20 pc.	Single i.p. injection with 0, 50 or 75 mg/kg bw	Not stated	The highest dose induced a small increase in non-viable implants and a strong increase in limb defects (syndactyly 21/39 versus zero in the control groups).
Hem68	Wistar rats (n=4/group)	Treatment on day 5, 9, 13, 14, 15, 16 or 20 post coitum. Dams were allowed to go to term.	Single i.p. injection with 0, 50, 75 (only day 16) or 100 (only day 16) mg/kg bw	Not stated	*50 mg/kg* induced a strong reduction of litter size when injected on day 5, 9 or 13 and a small reduction when injected on day 14 to 20. The higher doses of *75 and 100 mg/kg* induced stronger reductions on day 16 (average litter of 2.7 and zero resp.). *50 mg/kg* on day 16 induced an increase of tubules lacking germ cells in male offspring on day 1, 15 and 180 PN.
Hem68	Wistar rats (n=4/group)	Treatment (I) of dams on day 16 or 20 or (II) of male offspring on day 2, 4 or 7 post partum.	Single i.p. injection with 0 or 50 mg/kg bw	Not stated	Treatment on day 16 or 20 induced a reduction in gonocytes at birth and type a spermatogonia at day 15. Treatment on day 2, 4 or 7 induced a reduction in type a spermatogonia
Hem68	Wistar rats (n=4/group)	Treatment of dams on day 16. Dams were allowed to go to term.	Single i.p. injection with 0 or 50 mg/kg bw	Not stated	No effects on the fertility, body weight and seminal vesicles plus coagulating gland of the male offspring on day 120 pp. Small reduction in testes weight.
Hem69	Wistar rats (n=4 or higher per group)	Treatment of dams on day 13, 14, 15, 16 or 20. Offspring of tree litters were killed and ovaries of the females were examined on day 1, day 15 or 6 months PN. The remaining female offspring were tested for fertility after 4 months.	Single i.p. injection with 50 mg/kg bw	Not stated	Injection on day 16 had a strong destructive action on oogonia and resulted in elimination of germ cells from offspring. Ovaries from dams treated on day 20 appeared normal. Treatment of the dams on day 13 to 16, resulted in a strong decrease of fertility of the offspring (1 year old) (6/6, 6/6, 4/6, 5/6 females were sterile on day 13, 14, 15, 16 respectively). Treatment on day 20 did not affect the fertility of the offspring. The ovary weight of the offspring was reduced with 69% at 6 months after birth.

Annex **E**

Abbreviations

Abbreviations used:

bw	body weight
d	day
F	female(s)
i.p.	intraperitoneal
IPMS	isopropyl methanesulphonate
p.o.	per oss
i.v.	intravenous
M	male(s)
n	number
NOAEL	no adverse effect level
OECD	Organisation for Economic Cooperation and Development
PN	postnatal