Health Council of the Netherlands

Hexachlorophene

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

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Geachte staatssecretaris,

Graag bied ik u hierbij het advies aan over de effecten van hexachlorofeen op de vruchtbaarheid en het nageslacht; het betreft ook effecten die optreden na blootstelling via de borstvoeding. 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 raad.

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. L.J. Gunning-Schepers,
voorzitter

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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 State Secretary of Social Affairs and Employment

No. 2011/23, The Hague, October 19, 2011

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Contents

Samenvatting *9*

Executive summary *11*

1 Scope *13*
1.1 Background *13*
1.2 Committee and procedure *13*
1.3 Labelling for lactation *14*
1.4 Data *15*
1.5 Presentation of conclusions *15*
1.6 Final remark *15*

2 Hexachlorphene *17*
2.1 Introduction *17*
2.2 Human studies *18*
2.3 Animal studies *21*
2.4 Conclusion *27*

References *31*

Annexes *35*
A The Committee *37*
B Regulation (EC) 1272/2008 of the European Community *39*
C Additional considerations to Regulation (EC) 1272/2008 *51*
D Fertility and developmental toxicity studies *53*

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad hexachlorofeen onder de loep genomen. Hexachlorofeen wordt gebruikt als desinfectans in onder andere zeep en tegen bacteriegroei in medicijnen en cosmetica. 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 hexachlorofeen komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte humane gegevens zijn, maar dat voldoende diergegevens laten zien dat hexachlorofeen de fertiliteit niet schaadt. De commissie adviseert daarom om hexachlorofeen niet te classificeren

- voor effecten op ontwikkeling adviseert de commissie om hexachlorofeen in categorie 2 (*stoffen die ervan verdacht worden dat zij toxisch zijn voor de menselijke voortplanting*) te classificeren en met H361/d (*wordt ervan verdacht het ongeboren kind te schaden*) te kenmerken
- voor effecten tijdens lactatie, adviseert de commissie om hexachlorofeen niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report, the Health Council of the Netherlands reviewed hexachlorophene. Hexachlorophene is used as a disinfectant in soaps and as a preservative in drugs and cosmetics. 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 hexachlorophene, these recommendations are:

- for effects on fertility, the Committee recommends not classifying hexachlorophene on the basis of a lack of appropriate human data and sufficient animal data which show that classification is not indicated
- for effects on development, the Committee recommends classifying hexachlorophene in category 2 (*suspected human reproductive toxicant*) and labelling with H361/d (*suspected of damaging the unborn child*)
- for effects during lactation, the Committee recommends not labelling hexachlorophene 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 B and 2) and compounds with effects on or via lactation.

1.2 Committee and procedure

The present document contains the classification of hexachlorophene by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances. The members of the Committee are listed in Annex A.

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 B. 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 C).

In 2010, the President of the Health Council released a draft of the report for public review. No comments were received.

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 during 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 during 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 exceeds 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 Toxline, Medline, Toxcenter, SciSearch, and Chemical Abstracts starting from 1990 up to June 2005. A final search was performed in October 2010 in PubMed. Literature was selected primarily on the basis of the text of the abstracts. Literature before 1990 was taken from a monograph prepared for the Commission of the European Communities.[3] Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided into literature cited and literature consulted but not cited.

The Committee describes 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.

1.5 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 toxic to reproduction is indicated.

1.6 Final remark

The classification of compounds is based on hazard evaluation (Niesink *et al.*[19]) only, which is one of a series of elements guiding the risk evaluation process. The committee emphasizes that for derivation of health-based occupational exposure limits, these classifications should be placed in a wider context. For a

comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment, and recommendations of other organizations.

Chapter 2

Hexachlorphene

2.1 Introduction

name	:	hexachlorophene
CAS registry number	:	70-30-4
synonyms	:	phenol, 2,2'-methylenebis[3,4,6-trichloro-; 2,2'-methylenebis-(3,4,6-trichloro-phenol); bis(2-hydroxy-3,5,6-trichlorophenyl)methane; bis(3,5,6-trichloro-2-hydroxyphenyl) methane; 2,2'-dihydroxy-3,3',5,5',6,6'-hexachlorodiphenyl-methane; 2,2'-dihydroxy-3,5,6,3',5',6'-hexachlorodiphenylmethane; 2,2',3,3',5,5'-hexachloro-6,6'-dihydroxydiphenylmethane; hexachlorofen; hexachlorophane; hexachlorophen; hexophene; trichlorophene
appearance	:	white to light tan, crystalline powder; white, free-flowing powder
use	:	disinfectant in soaps, lotions, solutions, powders; preservative in drugs and cosmetics. In the EU, hexachlorophene is not permitted for use in cosmetics. In the Netherlands, it is an ingredient in over-the-counter products for treating excessive perspiration. According to the on-line database of the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), hexachlorophene is not permitted for use as an active substance in pesticides/biocides in the Netherlands. In the US, its use as a prescription drug is restricted to surgical scrubbing or hand washing as part of patient care and to topical application to control an outbreak of gram-positive infection in case other procedures have not been successful. In over-the-counter drugs and cosmetics, it may be used as a preservative in products other than those which in normal use may be applied to mucous membranes or which are intended to be used on mucous membranes, at levels that are in no event higher than 0.1%. Hexachlorophene is not mentioned among the chemicals on the pesticide re-registration list of the US Environmental Protection Agency (EPA)
molecular weight	:	406.9
molecular formula	:	$C_{13}H_6Cl_6O_2$
melting point	:	164-165°C; 166-167°C

vapour pressure	:	not found
solubility in water	:	not soluble (at 25°C: 0.01 g/100 mL)
Log $P_{octanol/water}$	:	7.54 (experimental); 6.92 (estimated)
conversion factor (at 20°C, 101.3 kPa)	:	not applicable
EU classification	:	Acute tox. 3/H311; Acute tox. 3/H301

Data from IARC[2], HSDB[18], and http://esc.syrres.com/interkow/interkow.exe.

Hexachlorophene is a neurotoxic substance inducing intramyelinic vacuoles and extensive oedema in the white matter of the brain of several species including humans after short-term and long-term exposure through oral and dermal routes. Clinical effects include paralysis of the hind limbs in animals and blindness in humans. Marked sensitivity of the optic nerve was noted.[3] In 1979, IARC[2] concluded that the available data did not allow an evaluation of the carcinogenicity of hexachlorophene to be made because only one acceptable animal study (negative) was available, while no human data were found.

2.2 Human studies

Fertility studies

There are no studies available regarding the effects of exposure to hexachlorophene on human fertility.

Developmental toxicity studies

Halling[9] compared the birth prevalence of severe malformations in neonates born to mothers working in hospitals where hexachlorophene-containing soap (Sanitvål; 0.5% hexachlorophene) or sudsing soapless cleanser (pHisoHex; 3.0% hexachlorophene) were used to the prevalence in neonates born to mothers working in the same hospitals without exposure to these products. Initially, data were used from two hospitals in the period from 1970 to 1976. Six severely malformed infants were born to 65 mothers with exposure to hexachlorophene, as well as six infants with less severe malformations. In the control group, only one hip dislocation was observed in 68 infants. Examination of the records revealed no apparent differences in age, infections, or drug use. Subsequently, a larger group of women working in similar units in four other hospitals was studied. The mothers in the exposed group were reported to have 20-60 hand washings per day. The control groups were not always from the same period, but usually from the period after discontinuation of the use of hexachlorophene

cleansing agents. In the six hospitals studied, a total of 25 infants with major malformations were born out of 460 births among exposed mothers compared to none out of 233 births among unexposed mothers. The numbers of malformations classified as minor were 46/460 among exposed compared to 8/233 among unexposed. The major malformations reported were: six anal or oesophagus atresia, three kidney malformations, one diaphragmatic hernia, four cleft lip and palate, five severe cardiac malformations, one omphalocele, one multiplex arthrogryposis, one eye malformation, one hand missing, one talipes equinovarus, and four major malformation of the central nervous system. The percentage of malformations found in the control group (3.4%) was comparable to the overall incidence of serious malformations in the general population of Sweden in that time period (3%).

In an editorial by Janerich[12], a relative risk of 4.4 (95% CI: 1.4-7.6) was calculated for major and minor malformations combined. The results were not corrected for confounders, such as age, parity, and smoking behaviour.

In a letter to the editor, Källén[22] disputed the design of the study by Halling, suggesting that inclusion of hospitals with a cluster of malformed infants led to selection bias. He also questioned the absence of major malformations among controls, stating that the total malformation rate, including major and minor malformations, could be as high as 6-7%.

In order to find out whether or not clusters of malformations as seen by Halling could be found in similar hospitals, Baltzar *et al.*[4] studied a cohort of approximately 1,500 women who worked in one out of 31 participating Swedish hospitals for chronic diseases during the latter part of pregnancy, but not necessarily during early pregnancy, and gave birth in the period 1965 to 1975. Hexachlorophene exposure was not characterized but the use of hexachlorophene-containing soaps was common in these hospitals. No increased prevalence of registered malformations was observed, except among a group of 200 women who worked in one of three hospitals (two in Gothenburg; one in Mölndal) and gave birth in 1973 or 1974. In this group, 13 children (versus four expected) were born with serious malformations resembling those initially described by Halling.

This finding led to an extended study including all 29,806 women working in medical occupations in Sweden who gave birth during the years 1973-1975. Information on these deliveries was obtained from the Medical Birth Record Register. The actual work status of the women was not well defined: only

approximately 80% might have worked for at least half of the pregnancy and exposure to hexachlorophene was not assessed. Compared to all women who delivered in Sweden, a statistically significant increase in perinatal death rate was observed in 1973, which could not be related to any specific occupational exposure. No statistically significant difference was observed for registered congenital malformations, although in each of the three years studied, the observed numbers were slightly higher (5.2%) than the expected (5.0%). The increased prevalence of malformations found in the two Gothenburg hospitals mentioned above was again observed (42/451; 29 expected for all registered malformations).

In order to determine whether or not perinatal mortality or malformation rates could be related to hexachlorophene-containing soaps, an inquiry was made to each hospital. From the replies, a number of hospitals with extensive use of these soaps could be identified as well as a number of hospitals with no or rare use. Between these groups, no differences were observed in the numbers of perinatal deaths or malformed infants for births occurring in 1973 or 1975. However, data for 1974, with the largest difference between observed and expected numbers of malformations, were not presented. Overall, no conclusions can be drawn from this study, given the huge potential for misclassification of both outcome and exposure.

Hemminki *et al.*[10] compared exposure to several agents between nurses who had a spontaneous abortion (217 cases) or a malformed child (46 cases) and nurses who had a normal birth (571 controls) between the years 1973 and 1979. The controls were matched for age and hospital of employment. Information was collected on the use of hexachlorophene-containing soaps and other agents during early pregnancy. The odds ratios for hexachlorophene exposure after controlling for the effects of confounding variables, were 0.9 (95% CI: 0.5-1.8) and 0.3 (95% CI: 0.1-1.8) for spontaneous abortion and malformed children, respectively. However, the low power of this study for congenital malformations limits its interpretation.

Källen *et al.*[14] performed a case-control study, as part of a cohort study on physiotherapists in Sweden in the period 1973-1978, on the difference in exposure to hexachlorophene in mothers giving birth to dead infants or infants with serious malformations (n=37) and two matched controls per case (matched for age, parity, and seasonality). Exposure information was based on questionnaires (response 93%). No difference was seen in exposure to

hexachlorophene between cases (42% often/daily) and controls (38% often/daily), but the small numbers again hamper the interpretability of this study.

Kurppa *et al.*[16] studied the association between congenital malformations and exposure to chemicals in Finland in a case-referent study using 140 pairs of cases and non-malformed control children matched for region and date of birth. Exposure to hand washing agents was found in 33 cases and 44 referents. Exposure to hexachlorophene was found in six cases and six referents, indicating no association.

Roeleveld *et al.*[20] performed an explorative case-referent study on parental occupational exposure comparing 306 mentally retarded children with unknown causes with 322 children with other congenital defects with known causes unrelated to parental exposure (referents) in the Netherlands between 1979 and 1987. Exposure estimates were based on interviews. Several potential confounding variables were checked and the odds ratios were adjusted when necessary. An odds ratio of 3.1 (90% CI: 1.0-9.7) was found for exposure to hexachlorophene/phenylphenol in late pregnancy (8/306 cases versus 3/315 referents). Because of the explorative character of the study, 90% confidence intervals were used. No increased odds ratios were found for exposure of the fathers or for exposure of the mothers in other periods of pregnancy.

Lactation

No studies are available regarding the effects of hexachlorophene on human lactation.

2.3 Animal studies

Fertility and developmental toxicity studies with hexachlorophene in laboratory animals are summarized in Annex D.

General introduction

In addition to the specific studies on fertility and development, some studies on the placental transfer are available showing that hexachlorophene or its metabolites reach the foetus.[6,5,15] These studies showed, amongst others, that following intramuscular (mice) or intravenous (monkey) injection of ^{14}C-

hexachlorophene on single days in early pregnancy (*i.e.* during organogenesis), radioactivity accumulated in tissues with a high cell proliferation rate, especially in embryonic neural epithelium, *e.g.* in the neural tube.[5,12]

Fertility studies

In a three-generation diet study performed by Schwartz[21], six-seven-week-old male and female rats received hexachlorophene at doses of 0, 10, 20, and 60 g/kg diet, which resulted in mean daily doses for the parental generations of approximately 0.5-0.9, 1.1-1.9, and 3.3-5.8 mg/kg bw, respectively. Twenty males and 40 females per group were treated for a 56-day pre-mating and a 15-day mating period, throughout gestation and during lactation. At weaning, the F1a pups were examined for external abnormalities and then selected for a 24-month oral toxicity study. The F0 parental animals were then reduced to 10 males and 20 females per group and not selected F1a pups en F0 parents were sacrificed. After a 10-day rest period, the selected F0 animals were mated to produce F1b litters. One week after weaning, 10 male and 20 female F1b pups per group were selected to comprise the second generation parental animals. All remaining F1 pups and F0 parents were sacrificed. The F1 parents were treated similarly to the F0 animals. However, they produced three litters: F2a, that was discarded, F2b, that was used to select the third generation parents, and F2c, that was used for uterine content examinations at gestational day 13 and 20 (Caesarean section). The F2 parents were treated similarly to the F0 en F1 parents. They produced two litters: F3a, that was discarded, and F3b. After weaning, five F3b pups and five F2 parents per sex per group were sacrificed and necropsied. The remaining F2 and F3b animals were examined for external abnormalities and then sacrificed and discarded.

Hexachlorophene treatment did not affect male and female fertility indices and length of gestational periods. Uterine content examinations performed in the F2c at gestational day 13 and 20 did not reveal statistically or biologically significant effects of hexachlorophene on the mean numbers of viable or non-viable implantations, total implantations, and corpora lutea at the low and mid dose. At the high dose, slight decreases in the mean numbers of corpora lutea and of total and viable implantations were observed at the examination on gestational day 13; at the examination on gestational day 20, there were slight increases in the mean numbers of corpora lutea and of total implantations. In the parental rats, hexachlorophene treatment did not induce changes in general behaviour, appearance, and survival. Apart from (not statistically significantly) lower body weights in all F1 and F2 treatment groups, group mean body weights did not

differ between groups. Post-mortem examinations of parental rats which died and of F2 parental rats did not show hexachlorophene-related macroscopic or microscopic lesions, but brains were not examined.

Gaines *et al.*[7] performed a two-generation diet study in rats (see *Developmental toxicity studies*) but did not present detailed data on fertility end points. However, from the number of pair matings at each dietary level for the F1a, F1b, and F2a generation, the committee infers that fertility was not affected.

James *et al.*[11] treated male rats orally (gavage) with a subneurotoxic dose of 5 mg/kg bw/day for four or nine weeks or nine weeks plus 13 weeks recovery. The serum hormone levels or the sex organs were not affected. A temporary reduction in all germ cells counted was found at four weeks but not at nine weeks.

James *et al.*[11] also treated male dogs orally (gavage) with a subneurotoxic dose of 3 mg/kg bw/day for nine weeks or nine weeks plus 13 weeks recovery. The serum hormone levels or the sex organs were not affected. A reduction in type B spermatogonia was found at nine weeks compared to the recovery animals. There were no concurrent controls.

Thorpe[22] gave male rats a single oral (gavage) dose of 125 mg/kg bw which induced mortality and diarrhoea but also a marked reduction in the sperm content of the epidydimides and abnormal spermatogonic cells in the seminiferous tubules. This effect was almost reversed on day 21. At 75 mg/kg bw, only diarrhoea was found and the same changes in the testes but to a lesser extent. No effects were found at 25 mg/kg bw.

Thorpe[22] also treated rats with five daily oral (gavage) doses of 75 mg/kg bw/day which induced diarrhoea, hindquarter weakness and reduced body weight gain. Furthermore, the epidydimides were almost devoid of sperm cells but contained degenerated cells of spermatoid origin and changes in the semi-niferous tubules. No effects were found at 25 mg/kg bw/day for five days.

Thorpe[23] administered single oral (gavage) doses of 25 or 50 mg/kg bw to rams. Treatment induced severe atrophy of the testes requiring approximately 21 days to develop. No information was provided on the systemic toxicity at these dose levels but two to four daily treatments with 50 mg/kg bw resulted in mortality or moribund animals.

Gellert *et al.*[8] washed the entire body, except the head, of male and female rat pups for 10 minutes with a commercial preparation containing 3% hexachlorophene on post-natal day 1 through 8. This induced tremors and ataxia in all treated rats and increased mortality rates (by 14 days of age: 25% in males; 13% in females vs. 0% in controls). Treatment did not affect the onset of puberty, oestrous cycle, and fertility in females when tested at 4.5 months of age. In males, there were statistically significant decreases in the number of siring progeny at seven months and in the number of matings at 9.5 months. At 11 months, the number of rats ejaculating was statistically significantly reduced but not mounting and intromission. Necropsy after 13 months showed a statistically significant increase in incidence and severity of prostate changes. Gellert *et al.* stated that the infertility of the adult males was the result of their inability to ejaculate. This may have been caused by a permanent disruption of the central nervous system-integrated ejaculatory reflex. According to Gellert *et al.*, the cause of the disruption may have been hexachlorophene or known contaminants of hexachlorophene preparations such as dioxins.

Alleva[1] gave male and female hamster pups single subcutaneous injections of doses of hexachlorophene of 0, 3, or 6 mg/kg bw on post-natal day 0, 1, 2, 4, or 12, or three injections of 3 mg/kg bw on post-natal day 0, 1, and 2. This caused some mortality when given on day 0, 1, and/or 2, but had no effect on fertility parameters.

Developmental toxicity studies

Gaines *et al.*[7] administered oral (gavage) doses of hexachlorophene of 0, 1, 5, 10, or 20 mg/kg bw/day to pregnant rats on gestational day 7 through 15. The females were allowed to litter and the pups were weaned and discarded at post-natal day 21. All pups appeared normal on post-natal day 21, and no stillbirths were observed. No effects on litter size, survival to weaning, and weight at weaning were seen at 10 mg/kg bw/day or lower. At the highest dose, 2/9 dams failed to produce litters while a third animal delivered only one pup. No information was provided on maternal effects but Gaines *et al.* stated that 25 mg/kg bw/day had been lethal to about 50% of female rats treated for 14 days. Malformations were not studied.

Gaines *et al.*[7] exposed groups of 10 rats per sex and dose in a two-generation diet study to 20 (1-2.5 mg/kg bw/day) and 100 ppm (5-12 mg/kg bw/day), from age 4-5 weeks (F0) through mating for F1a and F1b on day 54 and 166 of exposure

until necropsy on day 258. Animals were mated within their exposure groups. The F1a generation was sacrificed at weaning on post-natal day 21. The F1b generation was weaned on day 21, mated on day 95 (n=12) to produce F2a, and sacrificed on day 145. F2a rats were sacrificed at weaning on day 21. Body weight gain of the breeders was not affected and no sign of leg weakness was found. Small vacuolated areas in the white matter of the brain were found in 7/10 males and 3/10 females in the F0 at the highest dose level. In the F1b generation, this effect was only found in three females. No indications for parental toxicity were found at 20 ppm. Number of litters born and litter size were not affected by treatment. Survival until weaning was statistically significantly reduced in the F1b (62.4% vs. 98.6%), reduced in the F1a (80.0% vs. 96.0%), and unchanged in the F2a (92.1% vs. 97.6%) at the highest dose level. Body weight at weaning was not affected. Small vacuolated areas in the white matter of the brain were found in 7/19 pups from four litters of the F1b weanlings fed 100 ppm but not in 30 pups from 6 F2a litters fed 100 ppm.

In the three-generation diet study by Schwartz (see *Fertility studies*), hexachlorophene treatment did not affect the viability and survival of the pups through weaning. Pup body weights did generally not differ between groups. Body weights of the F3a pups of the mid-dose group at lactational day 21 were statistically significantly increased. Body weights of the F1a and F2b pups at the high dose on lactational day 0, 4, 14, and 21 were slightly lower when compared to controls, as were the body weights of the F1b, F3a, and F3b pups of the high-dose group at lactational day 21. With respect to general behaviour and appearance, no changes were seen between the litters in the treated and control groups. Examinations performed in the F2c at gestational day 13 did not reveal differences in the mean numbers of early resorptions and post-implantation losses between groups. Examination of the F2c at gestational day 20 did not show differences in foetal sex ratio and foetal body weights when treated groups were compared with controls, or in the mean numbers of early resorptions, post-implantation losses, and viable and non-viable foetuses comparing the low- and mid-dose groups with controls; at the high dose, there were slight increases in the mean numbers of viable and non-viable foetuses and slight decreases in the mean numbers of early resorptions and post-implantation losses. Apart from a statistically significant increase in the numbers of foetuses with malformations (mainly cartilage anomalies) in the mid-dose group, no malformations or soft tissue or skeletal alterations were observed. Post-mortem examinations of the F3b offspring of the high-dose group did not show hexachlorophene-related gross pathologic or microscopic lesions; brains were not examined.[21]

Thorpe[22] exposed pregnant rats throughout pregnancy to a diet containing 0.1 and 0.05% hexachlorophene. This did not result in embryotoxicity or teratogenicity at examination on day 20.

Thorpe[22] exposed pregnant rats on gestational day 8 to a single dose of 50 or 100 mg/kg bw by gavage. This resulted in maternal mortality (2/6) at 100 mg/kg but not in embryotoxicity or teratogenicity. No effects were found at the lower dose at examination on day 20.

Majumdar *et al.*[17] injected mice subcutaneously with 12.5 or 25 mg/kg bw/day on gestational days 3-8, 7-12, or 11-16. No information was provided on the maternal toxicity of the tested dose levels. However, it was stated that a dose of 50 mg/kg bw induced 70-90% mortality. Except for the low dose administered during gestational day 11-16, all treatments induced an increase in resorbed and dead foetuses and a decrease in live foetuses on gestational day 19 when the females were sacrificed. Foetuses were only checked for external malformations.

Kimmel *et al.*[15] treated pregnant rats intravaginally with 0, 20 (suspension or commercial product containing 3%), 80 (suspension 12%), or 300 mg/kg bw/day (suspension 45%) on gestational day 7, 8, 9, and 10 which resulted in maternal mortality (2/12), weight loss, severe diarrhoea, vaginal ulceration (day 20), and vaginal infection at 300 mg/kg bw, and weight loss, diarrhoea, vaginal infections, and weakness at 80 mg/kg. Only one case of microscopic changes of the maternal brain was seen (dose level not stated). A statistically significant increase in dead or resorbed foetuses (33% vs. 8%) was found, and the foetal weight per litter was statistically significantly reduced at 300 mg/kg bw. A statistically significant increase in malformed foetuses (19% and 40% vs. 4%) was found at 80 and 300 mg/kg bw. Frequently produced abnormalities were anophthalmia, microphthalmia, hydrocephalus, wavy ribs, and urogenital defects. The mothers with the most severe toxicity had the litters with the most severe effects. No effects were seen at 20 mg/kg bw. The uptake and distribution of hexachlorophene was compared at 24 hours after an oral, intravaginal, or dermal exposure of dams on gestational day 11 to approximately 150 mg/kg bw. The radioactivity in most organs after oral exposure was in the same range as after intravaginal exposure with exception of the vagina. The levels in the embryo were also comparable (vaginal: 4.1±0.8 µg/g; oral: 5.7±0.5 µg/g). The radioactivity level after dermal exposure was clearly lower in all organs. However, the embryo levels (4.0±0.8 µg/g) were comparable.

Lactation

Gaines *et al.*[7] (see *Developmental toxicity studies*) estimated the concentrations of hexachlorophene in the milk of lactating rats given dietary doses of hexachlorophene of 20 (1-2.5 mg/kg bw/day) and 100 ppm (5-12 mg/kg bw/day). From stomach content samples obtained from three-four-day-old pups, the amounts of hexachlorophene were determined to be 0.07 and 0.33 mg/kg milk, respectively.

2.4 Conclusion

No information is available on the effects of hexachlorophene on fertility in humans.

In the three-generation study by Schwartz[21] in which mean daily doses of hexachlorophene of approximately 0.5-0.9, 1.1-1.9, and 3.3-5.8 mg/kg bw were administered to F0, F1, and F2 parental rats through the diet, no consistent, relevant effects on male and female fertility indices and length of gestational periods were observed in any of the generations. In this study, no effects on parental animals were observed. In studies in which hexachlorophene was administered by gavage to male rats, male dogs, or rams, effects on fertility end points were either of doubtful significance[11] or were caused at dose levels inducing or expected to induce general toxicity.[1,22,23]

Overall, based on the data from animal studies, the committee is of the opinion that sufficient data show that no classification for the effects of hexachlorophene on fertility is indicated.

Halling[9] reported an increased birth prevalence of malformed infants in hospital staff exposed to hexachlorophene in several hospitals in Sweden. However, this study was criticized by Källen[13] because of methodological issues. In a few other studies, no association was observed between congenital malformations and hexachlorophene exposure, but these studies were underpowered.[10,14,16] In a large register-based study, Baltzar *et al.*[4] did not find an association between the registered occurrence of malformed infants and working in hospital or exposure to hexachlorophene either, but adequate exposure and outcome assessment was lacking. In an explorative case-referent study[20], an association was found between mental retardation in children and maternal exposure to hexachlorophene/phenylphenol in the last three months of pregnancy using a 90% confidence interval.

In conclusion, the Committee is of the opinion that the available human data do not provide sufficient evidence for a causal relation between human exposure to hexachlorophene and subsequent developmental toxic effects in offspring.

Regarding animal studies, in the three-generation study by Schwartz[21] in which mean daily doses of hexachlorophene of approximately 0.5-0.9, 1.1-1.9, and 3.3-5.8 mg/kg bw were administered to F0, F1, and F2 parental rats through the diet, slightly lower body weights were observed in the high-dose groups of F1a and F2b pups on lactational days 0, 4, 14, and 21 and of F1b, F3a, and F3b pups on lactational day 21. Uterine content examination of the F2c at gestational day 20 revealed slight increases in the mean number of viable and non-viable foetuses and slight decreases in the mean number of early resorptions and post-implantation losses at the high dose. Apart from a statistically significant increase in the number of foetuses with malformations (mainly cartilage anomalies) in the mid-dose group, no malformations or soft tissue or skeletal alterations were seen. No effects on parental animals were observed. In the two-generation study by Gaines *et al.* with dietary doses of 1-2.5 and 5-12 mg/kg bw/day, there was a statistically significant decrease in pup survival at weaning in the high-dose F1b group and a decrease in the high-dose F1a group. There were no signs of toxicity in parental animals, but post-mortem examination showed small vacuolated areas in brain white matter in 7/10 F0 males and 3/10 F0 females and in 3/10 F1b females of the high-dose groups. The studies by Thorpe[22], showing no effect of exposure to 0.05 and 0.1% hexachlorophene in the diet during pregnancy on pregnancy and development, were performed with a small number of animals (n=3-4) and were poorly reported. Increases in malformations including microphthalmia and anophthalmia were found by Kimmel *et al.*[15] at two dose levels after intravaginal exposure which also induced maternal toxicity. At the high dose, there was also an increase in dead or resorbed foetuses and reduced foetal weight.[8,17]

Overall, developmental effects were found in multigeneration studies at dose levels which induced histological lesions in the brain, if examined, in line with those induced by hexachlorophene in subchronic general toxicity studies.

Therefore, based on the data from animal studies, the committee proposes to classify hexachlorophene in category 2 (‘suspected human reproductive toxicant’) and to label with H361/d.

Transfer of hexachlorophene in milk was shown in a rat study.[7] The highest value was approximately 0.3 mg/kg. Effects were found in studies with exposure during the lactation period. In two- and three-generation studies[7,21], the survival to weaning was statistically significantly reduced in some generations. The effects seen during lactation could either be via lactation or caused by the prenatal exposure through the mother. The available data do not allow the committee to distinguish between these possibilities.

Overall, the Committee proposes not labelling hexachlorophene for effects during lactation because of a lack of appropriate data.

Proposed classification for fertility

Lack of appropriate human data precludes the assessment of hexachlorophene for fertility, while sufficient animal data show that no classification for hexachlorophene is indicated for effects on fertility.

Proposed classification for developmental toxicity

Category 2; H361/d.

Proposed labelling for effect during lactation

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

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

Annexes

Annex **A**

The Committee

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The first draft of the present document was prepared by J.J.A. Muller (National Institute for Public Health and the Environment (RIVM), Bilthoven, 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 President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

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.

Effect 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

developmental 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 calculation 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.

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

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 for effects 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 lactation
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 foreffects 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 **C**

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 B. 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 B, 3.7.2.2.1.).
- Adverse effects in a reproductive study, reported without information on 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 does 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 **D**

Fertility and developmental toxicity studies

Table 1 Fertility studies in animals.

authors	species	experimental period/ design	dose/route	general toxicity	effects on reproductive organs/effects on reproduction
Schwartz (1979)	Charles-River CD rats (F0: n=20 males; 40 females/group; F1, F2: n=10 males; 20 females/ group	3-generation study: F0 treated for a 56-d pre-mating and a 15-d mating period (1:2), throughout gestation and lactation; 10 d after weaning F1a, 10 males and 20 females selected for mating (1:2) for F1b; when 100 d old, F1b generation mated for F2a and then for F2b and F2c; F2b similarly mated for F3a and F3b; at weaning, F1a pups examined for external abnormalities (then selected for a 24-mo oral toxicity study); after weaning, 5 F3b pups and 5 F2 parents per sex per group sacrificed and necropsied; remaining F2 and F3b animals examined for external abnormalities only	0, 10, 20, 60 g/kg diet (*i.e.*, F0, F1, F2 males: 0, 0.5-0.6,1.1-1.3, 3.3-3.8 mg/kg bw/d; F0, F1, F2 females: 0, 0.9, 1.9, 5.6-5.8 mg/kg bw/d)	no changes in general behaviour, appearance and survival in parents; no effect on mean bw, apart from (not statistically significantly) lower bw in all F1 and F2 treatment groups; at post-mortem examinations of parental rats which died and of F2 parental rats: no macroscopic or microscopic lesions (brains not examined)	no effect on male and female fertility indices and length of gestational periods; at uterine content examinations in F2c at gd 13 and 20: no statistically or biologically significant effects on the mean number of viable or non-viable implantations, total implantations, and corpora lutea at the low and mid dose; at the high dose: slight decreases in the mean number of corpora lutea and of total and viable implantations at gd 13; at gd 20: slight increases in the mean number of corpora lutea and of total implantations

James *et al.* (1980)	male Sprague-Dawley rats (n=15/group)	4 or 9 wk; 9 wk plus 13 wk recovery (number of d/wk not indicated)	0, 5 mg/kg bw/d; gavage	subneurotoxic dose	no effect on serum hormones and sex organs, only a temporary reduction in all germ cell types counted at wk 4 but not at wk 9
James *et al.* (1980)	male beagle dogs (n=4/group); no controls	9 wk; 9 wk plus 13 wk recovery (number of d/wk not indicated)	3 mg/kg bw/d; gavage	subneurotoxic dose	no effect on serum hormones and sex organs, only a decrease in Type B spermatagonia compared to recovery animals
Thorpe (1967)	male Wistar rats (n=6/group)	single treatment with sacrifice at d 1, 2 , 5, 12, and 21 after dosing	0, 25, 75, 125 mg/kg bw; gavage	125 mg/kg: mortality and diarrhoea 75 mg/kg: diarrhoea 25 mg/kg: no effects	125 mg/kg: marked reduction in the sperm content of the epidymis; increased percentage of abnormal spermatogonic cells in the seminiferous tubules from day 2; almost complete recovery on d 21; 75 mg/kg: comparable but less extensive changes; 25 mg/kg: no effects
Thorpe (1967)	male Wistar rats (n= 6/ group)	5 d/wk; sacrifice on d 5	0, 25, 75 mg/kg bw/d; gavage	75 mg/kg: diarrhoea, hindquarter weakness, reduced bw gain; 25 mg/kg: no effects	75 mg/kg: epididymis devoid of sperm cells but with degenerated cells of spermatid origin and changes in the seminiferous tubules 25 mg/kg: no effects
Thorpe (1969)	rams (n=3 (control) or 6)	single treatment with unilateral orchidectomy on post-treatment d 2, 7, 21	0, 25, 50 mg/kg bw; gavage	no information provided but 4 doses of 50 mg/kg bw were lethal or resulted in moribund rams	50 mg/kg; d 2: increase in proportion of seminiferous tubules containing only Sertoli cells, primary spermatocytes, and a few maturing spermatids; d 7: extensive focal degeneration of spermatogenic cells and other changes; reduction of sperms in epididymis; increase in spermatid derived round cells; d 21: many tubules with Sertoli cells only; no sperms in epididymis 25 mg/kg; d 7: less severe effects; d 21: comparable effects

Gellert *et al.* (1978)	Sprague-Dawley rats (n=15/sex; controls=7-8)	pnd 1-8; section after 13 mo	3% solution; dermal, *i.e.*, body of pups, except head, washed for 10 min; untreated control group and a group treated with cleanser not containing hexachlorophene included	ataxia and tremors in all treated rats by d 9; mortality by d 14 was 25% in males and 13% in females; these rats showed severe vacuolation of the myelin	no effect on onset of puberty, oestrous cycles, fertility in females; statistically significantly reduced fertility in males at 7 and 9.5 mo; significantly reduced number of rats ejaculating at 11 mo but no effect on mounting and intromission; severe changes of the prostate (cysts, squamous metaplasia, fibrosis) in 7/11 treated rats compared to mild changes in 3/14 control rats; abundant motile sperm found in all epididymal smears
Alleva, (1973)	hamster (Lakeview)	single treatment of pups on pnd 0, 1, 2, 4, or 12	0, 3, 6 mg/kg bw; sc	mortality; no other effects studied.	no effects on time of puberty, oestrous cycle regularity, fertility or bw indicating no effect on the sexual differentiation of the hypothalamic centre

n=number; bw = body weight; d=day(s); wk=week(s); min=minutes; mo=month(s); gd=gestational day(s); pnd=post-natal day(s); sc=subcutaneous.

Table 2 Developmental toxicity studies in animals.

authors	species	experimental period/ design	dose/route	general toxicity	developmental toxicity
Gaines *et al.* (1973)	Sherman rats (n= 8-10/group)	gd 7-15; pups reared until pnd 21	0, 1, 5, 10, 20 mg/kg bw/d; gavage	no information provided; about 50% mortality at 25 mg/kg bw/day for 14 d	malformations not studied 20 mg/kg bw/day: 2/9 dams failed to produce litter; ≤10 mg/kg bw/day: no effects
Schwartz (1979)	Charles-River CD rats (F0: n=20 males; 40 females/ group; F1, F2: n=10 males; 20 females/group	3-generation study: see above Table 1; F2c used for uterine content examinations at gd 13 and 20 (Caesarean section)	0, 10, 20, 60 g/kg diet (*i.e.*, F0, F1, F2 males: 0, 0.5-0.6,1.1-1.3, 3.3-3.8 mg/kg bw/d; F0, F1, F2 females: 0, 0.9, 1.9, 5.6-5.8 mg/kg bw/d)	see above Table 1	no effect on pup viability and survival through weaning; generally, no effect on pup bw; statistically significantly increased bw of the F3a pups of the mid-dose group at pnd 21; slightly lower bw of the F1a and F2b pups at the high dose at pnd 0, 4, 14, and 21, and of the F1b, F3a, and F3b pups of the high-dose group at pnd 21; no changes with respect to general behaviour and appearance between the treated and control litters; at examinations of F2c at gd 13: no effects on mean number of early resorptions and post-implantation losses between groups; at examinations of F2c at gd 20: no effect on foetal sex ratio and foetal bw when treated groups were compared with controls, or in mean number of early resorptions, post-implantation losses, and viable and non-viable foetuses comparing the low- and mid-dose groups with controls; at the high dose, slight increases in the mean number of viable and non-viable foetuses, slight decreases in mean number of early resorptions and post-implantation losses,

					apart from a statistically significant increase in the number of foetuses with malformations (mainly cartilage anomalies) in the mid-dose group, no malformations or soft tissue or skeletal alterations; at post-mortem examinations of F3b offspring of the high-dose group: no macroscopic or microscopic lesions (brains not examined)
Kimmel *et al.* (1974)	Charles River rats (n=12)	gd 7, 8, 9, 10; sacrifice on gd 20.	0, 20, 80, 300 mg/kg bw; intravaginal	300 mg/kg: mortality (2/12) and vaginal ulceration; 80 and 300 mg/kg: diarrhoea, vaginal infections, bw loss	300 mg/kg: increased number of dead or resorbed (33 vs. 8%) and malformed foetuses (40 vs. 4%); reduced foetal 80 weight/mg/kg: increased number of malformed foetuses (10 vs. 4%); main abnormalities: microphthalmia, hydrocephalus, wavy ribs, urogenital defects
Majumdar *et al.* (1975)	mice (n=20)	gd 3-8, 7-12, 11-16; sacrifice on gd 19.	0, 12.5, 25 mg/kg bw/d; sc	not stated but 50 mg/kg bw/day induced 70-90% lethality	all treated groups, except low dose at gd 11-16: decreased number of live foetuses; increased number of resorbed and dead foetuses; no external malformations
Thorpe (1967)	Wistar rats (n=3 or 4)	gd 1-19; sacrifice on gd 20	0, 0.05, 0.1% in diet	not described	no embryotoxic or teratogenic effects
Thorpe (1967)	Wistar rats (n=5 or 6)	gd 8; sacrifice on gd 20	0, 50, 100 mg/kg bw; gavage	mortality at 100 mg/kg bw	no embryotoxic or teratogenic effects
Gaines *et al.* (1973)	Sherman rat F0: n=10 and F1b: n=12/sex/ group	2-generation study; rats exposed 54 and 166 days before mating for F1a and F1b and 95 days before mating for F2a	0, 20, 100 mg/kg diet; *i.e.*, 1-2.5, 5-12 mg/kg bw/d	no mortality, reduced bw or leg weakness; at 100 ppm brain lesions in F0 animals and F1b females	100 ppm: brain lesions in some F1b pups (day 21) not in F2a pups; reduced survival to weaning in F1a and F1b (significant) but not in F2a

n=number; bw = body weight; d=day(s); wk=week(s); mo=month(s); gd=gestational day(s); pnd=post-natal day(s); sc=subcutaneous.