Cyclohexanol

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 'Cyclohexanol'
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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 cyclohexanol. 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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Cyclohexanol

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. 2004/09OSH, The Hague, September 9, 2004

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad cyclohexanol onder de loep genomen. Cyclohexanol is een kleurloze vloeistof, dat met name gebruikt wordt als intermediair bij de synthese van nylon en kunststoffen. 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 cyclohexanol komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om cyclohexanol te classificeren in categorie 3 *(stoffen die in verband met hun mogelijke voor de fertiliteit schadelijke effecten reden geven tot bezorgdheid voor de mens)* en met Xn:R62 te kenmerken.
- voor effecten op de ontwikkeling adviseert de commissie cyclohexanol niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten tijdens de lactatie is de commissie van mening dat er onvoldoende gegevens zijn om cyclohexanol te kenmerken.

Executive summary

In the present report the Health Council of the Netherlands reviewed cyclohexanol. Cyclohexanol is a colourless solvent which is among other things used as a intermediate for the production of nylon and plasticizers. 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 cyclohexanol are:

- for effects on fertility, the committee recommends to classify cyclohexanol in category 3 *(substances which cause concern for human fertility)* and to label with Xn:R62.
- for developmental toxicity, the committee recommends not to classify cyclohexanol due to a lack of appropriate data
- the committee is of the opinion that a lack of appropriate data precludes the labelling of cyclohexanol for effects during lactation.

Chapter 1

Scope

1.1 Background

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

1.2 Committee and procedure

The present document contains the classification of cyclohexanol 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 Drs MM Tegelenbosch-Schouten and Ir. DH Waalkens-Berendsen at the Department of Target Organ Toxicology of TNO Nutrition and Food Research, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development and lactation of the above mentioned compound.

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

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

In 2004, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

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

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

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

Consequently, a substance should be labeled 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, e.g. the acceptable daily intake (ADI).

1.5 Data

Literature searches were conducted in the on-line databases Current Contents and Medline, starting from 1966 up to 2003 and by searches on internet. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted as

* Organisation for Economic Cooperation and Development

well as several websites regarding (publications on) toxicology and health. References are divided in literature cited and literature consulted but not cited.

The committee choose to describe both 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 characterisation, human exposure assessment and recommendations of other organisations.

* for definitions see Tox95

Chapter 2

Cyclohexanol

2.1 Properties

Name	:	Cyclohexanol
CAS-no	:	108-93-0
Use	:	Cyclohexanol is a basic industrial chemical and solvent. It is primarily used, either isolated or as a mixture, in the production of nylon intermediates. It is also used as intermediate for agricultural chemicals, plasticizers, rubber chemicals and pharmaceuticals. Very limited use as a special process solvent has been described.
Mol weight	:	100,16
Chem formula	:	$C_6H_{12}O$
Acute toxicity	:	Low to moderate single-dose toxicity (Dut90; IHF01): - oral LD_{50} in rats of 1500 - 2060 mg/kg - dermal LD_{50} in rabbits of 501 – 794 mg/kg
Toxicokinetics	:	Readily absorbed, subsequently metabolized, and excreted in the urine as glucuronides and sulfates within several days (IHF01)
R-phrases	:	R20/22 Harmful by inhalation and if swallowed Irritating to respiratory system and skin

2.2 Human studies

Fertility

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

Developmental studies

No studies were found regarding the effects of exposure to cyclohexanol on human development.

Lactation

No studies were found concerning the excretion of cyclohexanol in human breast milk.

2.3 Animal studies*

Fertility

No data concerning the potential of cyclohexanol to cause effects on female fertility were available. Relevant animal studies concerning male fertility were present, but of poor quality. A standard multigeneration reproductive study was not performed.

Tyagi *et al* administered subcutaneously a daily dose of 15 mg/kg bw/day cyclohexanol to adult male gerbils and rats for a period of 21 and 37 days, respectively (Tya79). Cyclohexanol caused a significant reduction in the weights of the testes, epididymides, seminal vesicle and ventral prostate, histopathological changes in these organs, and altered biochemistry in both species. The marked degenerative changes in the seminiferous tubules consisted of loss of type A spermatogonia, spermatocytes, spermatids and spermatozoa. Shrinkage of the seminiferous tubules and of the Leydig cells were also observed. Sertoli cells displayed varying degrees of vacuolation of the cytoplasm. The lumen of the epididymis and ductus deferens was empty and a few tubules showed the presence of degenerating cells. Total protein, RNA and sialic acid contents of the testes,

* To obtain more information on possible harmful effect of cyclohexanol the Industrial Health Foundation Committee submitted a test plan for cyclohexanol to be used in the High Production Volume (HPV) Challenge program. Several proposals for GLP studies were presented (IHF01).

epididymis and seminal vesicle were lower in cyclohexanol treated rats and gerbils as compared to controls. Furthermore, the glycogen content in the testes was reduced. Testicular cholesterol and alkaline phosphatase were elevated. Serum analysis revealed normal functioning of liver, kidney and metabolic activities. Cyclohexanol treatment caused no changes of the level of serum proteins, cholesterol, blood sugar, urea and serum transaminase. Alkaline phosphatase activity, however, was significantly increased in treated gerbils. Blood parameters were considered normal, indicative for normal functioning of liver, and kidney.

From the results of this study, the committee concluded that in cyclohexanol treated gerbils and rats, the spermatogenesis was arrested at the level of primary spermatocytes. Recovery was not investigated. The study was reported very briefly. Data on the test compound (purity), dosing method (volume, site of injection) and in-life parameters (body weight, clinical signs) were not provided (Tya79).

Cyclohexanol was administered orally to two groups of male rabbits (n=5 per group) at a daily dose of 25 mg/kg bw/day for a period of 40 days. After 40 days, one group was allowed to recover for 70 days. In addition, a control group was included.

Compared to the control group, significant reductions in relative testes and epididymal weights were found in the treated rabbits. Microscopic examination of the testes presented marked degenerative changes, which consisted of a loss of type A spermatogonia, spermatocytes, spermatids and spermatozoa and morphological changes in spermatids. Leydig cells were shrunken. The lumen of the epididymis and ductus deferens was empty and a few tubules showed the presence of degenerating cells. Testicular and epididymal contents of protein, RNA, sialic acid, glycogen, and acid phosphatase were significantly reduced in treated animals, as was adrenal ascorbic acid content. Serum analysis of animals demonstrated significant increases in cholesterol, transaminase activity (SGPT), phospholipids, triglycerids, alkaline phosphatase activity and bilirubin. Serum protein was significantly decreased in this group, while acid phophatase activity, blood sugar, and urea levels were unchanged.

A marked recovery of the effects was seen after a 70-day recovery period. Relative testicular and epididymal weights did not completely reach control values, but were significantly higher than those of the treated animals, which were not allowed to recover. Along with organ weights, the seminiferous tubules and Leydig cells were restored to normal. Biochemical endpoints for reproductive tissues showed at least some recovery as compared to the treated group (most values were restored to control values). Significant differences in the recovery group compared to the control group were seen for cholesterol, phospholipids, alkaline phosphatase and bilirubin.

However, the study was reported briefly. Data on the test compound (purity), dosing method (means of oral administration) and in-life parameters (body weight, clinical signs) were not mentioned (Dix80).

Lake *et al* studied the effects on fertility of discyclohexyl phthalate (DCHP) and two of its metabolites, ie. cyclohexanol and monocyclohexyl phthalate (MCHP). Cyclohexanol was given daily by gavage to twelve 30-day old Sprague Dawley rats at a dose of 455 mg/kg bw for seven days. Control animals were given 5 ml corn oil/kg body weight by gavage. Animals were sacrificed for evaluation at 24 hours following the last dose. Relative liver weights were significantly increased, as were the activities of hepatic biphenyl 4-hydroxylase, 7-ethoxycoumarin o-deethylase, and aniline 4-hydroxylase and cytochrome p-450 content. There was no effect of treatment on relative kidney or testes weights. There was no mention of a histological evaluation of testicular tissue from cyclohexanol-treated animals (Lak82).

Developmental studies

The available data on the potential developmental toxicity of cyclohexanol consist of one study conducted in mice.

Cyclohexanol was given to mice of different strains (TB and NMNR) in a concentration of 1% as a dietary additive (estimated amount of 1200 mg/kg bw/day) during mating, gestation and lactation. An increase in mortality was seen at 21 days of life in TB mice (14.1% versus 11.9% - no statistics performed) and in NMRI mice (43.1% versus 12.2%). Pup mortality and growth however, were not evaluated until postnatal day 21. Treatment was continued with the offspring of the TB strain and the mortality of the second generation increased to 53.5% (no control data available). A slight inhibition of growth continued in successive generations when administration was continued, but stopped when administration was interrupted (Gon72).

Lactation

The only data available were the studies in mice described above (Gon72). In these studies pup mortality and growth were not evaluated until postnatal day 21.

2.4 Conclusion

No studies on the effects of cyclohexanol on human fertility were available. Only limited information is available on the effects on fertility from animal studies, mainly per-

formed in male animals. Although subchronic toxicity studies were performed, reproductive endpoints were not evaluated (Tre43a,b). A standard multigeneration reproductive study was not performed.

Two studies reported adverse effects on the male reproductive system (Tya79; Dix80). After oral exposure to cyclohexanol (only one dose tested) daily, for 40 days, Dixit *et al* found an effect on testis weight and sperm parameters in the absence of an effect on body weight. Tyagi *et al* treated male gerbils and rats subcutaneously (less relevant route of exposure), daily, for 21 and 37 days respectively (only one dose was tested). The findings of both studies were in agreement, despite the use of different species and a different route of exposure. However, the general toxicity in both studies was only reported briefly and only one dose was tested. A third study conducted in male rats (Lak82) did not find evidence for a change in testes weights after exposure to cyclohexanol by gavage for seven days. The duration of this study was, however, very short.

In conclusion, based on the findings by Dixit *et al*, the committee recommends classifying cyclohexanol in category 3 (substances which cause concern for human fertility') and labeling the compound with R62 ('possible risk of impaired fertility').

No studies on the effects of cyclohexanol on human development were available. A study conducted in mice indicated an adverse effect of cyclohexanol on pup postnatal growth (measured on postnatal day 21) when treatment was continuous from prior to conception, throughout gestation, lactation and the postweaning period (Gon72). Pup mortality and growth, however, were not evaluated until postnatal day 21. For that reason, the committee recommends not classifying cyclohexanol with respect to developmental toxicity because of a lack of appropriate data.

No publications concerning the excretion of cyclohexanol in human or animal milk were available. Only the studies in mice (Gon72) were available: In these studies pup mortality was not evaluated until postnatal day 21. Therefore, the committee recommends not labelling cyclohexanol for effects during lactation due to a lack of appropriate data.

Proposed classification for fertility

Category 3: R62.

Proposed classification for developmental toxicity

Lack of appropriate data precludes the assessment of cyclohexanol for development.

Proposed labelling for effects during lactation

Lack of appropriate data precludes the assessment of cyclohexanol for labeling for effects during lactation.

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Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
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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 2004 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 with cyclohexanol.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Tyagi *et al*. , 1979	Male gerbils (N=20/ group) Control group included	21 days exposure; haematological and histological examination 24 h after the last dose	15 mg/kg/ day, s.c.	- No effect on body weight, thyroid weight or adrenal weights - No effect on serum protein, chlolesterol, blood sugar, urea, or serum transaminases - Increased serum alkaline phosphatase - No effect on haematological parameters	- Decreased weights of testes, epididymis, seminal vesicle and ventral prostate - Degeneration seminiferous tubules, loss of sperm precursors, abnormal Sertoli and Leydig cells - Decreased protein, RNA, and sialic acid in testes, epididymis, and seminal vesicle - Decreased testicular glycogen - Increased testicular chlolesterol and alkaline phosphatase
	Male rats (N=20/ group) Control group included	37 days exposure; haematological and histological examination 24 h after the last dose	15 mg/kg/ day, s.c.	- No effect on body weight, thyroid weight or adrenal weights - No effect on serum protein, chlolesterol, blood sugar, urea, or serum transaminases - No effect on haematological parameters	- Decreased weights of testes, epididymis, seminal vesicle and ventral prostate - Degeneration seminiferous tubules, loss of sperm precursors, abnormal Sertoli and Leydig cells - Decreased protein, RNA, and sialic acid in testes, epididymis, and seminal vesicle - Decreased testicular glycogen - Increased testicular chlolesterol and alkaline phosphatase

Table 1.2 Fertility studies in animals with cyclohexanol.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Dixit *et al*. , 1980	Male rabbits (N=5/group); Control group (1), treated group with recovery (2) and treated group without recovery (3)	40 days exposure; haematological and histological examination 24 h after the last dose (groups 1 and 3) or after a recovery period of 70 days after treatment (group 2)	25 mg/kg/day p.o.	- No differences in autopsy body weight or relative adrenal weights - No histological evidence of liver necrosis Group 3: - Decreased adrenal ascorbic acid - Increased serum cholesterol, SGPT, phospholipids, triglycerids, alkaline phosphatase and bilirubin - Decreased serum protein - No change in acid phosphatase, blood sugar, or urea Group 2: - Most endpoints recovered to normal levels - Elevated serum cholesterol, phospholipid, alkaline phosphatase and bilirubin	Group 3: - Reduced relative testes and epididymis weights - Degeneration seminiferous tubules, loss of sperm precursors, abnormal Sertoli and Leydig cells - Reduced testicular and epididymal protein, RNA, sialic acid, glycogen, and acid phosphatase Group 2: Marked recovery of most endpoints to control, or near-control values
Lake *et al*., 1982	Male rats (N=12/group) Control group included	7 days exposure	455 mg/kg/day p.o.	- No effect on kidney weight - Increased relative liver weight; evidence for induction of certain parameters of xenoniotic metabolism	No effect on testes weight

Table 2 Developmental toxicity studies in animals with cyclohexanol.

Authors	Species	Experimental period/ design	Dose	Effects on reproductive organs/ effects on reproduction
Gondry, 1972	Mice (strain: TB and NMRI)	Treatment as dietary additive during mating, gestation and lactation of several generations	1% as dietary additive = approx. 1200 mg/kg/day	An increase in mortality during the first 21 days of life in TB mice (14.1% versus 11.9% - no statistics performed) and in NMRI mice (43.1% versus 12.2%). Treatment was continued with the offspring of the TB strain and the mortality of the second generation increased to 53.5% (no control data available). A slight inhibition of growth continued in successive generations when administration was continued, but stopped when administration was interrupted

Annex **E**

Abbreviations

Abbreviations used:

p.o.	Orally
s.c.	Subcutaneous
N	Number
LD	Lethal dose
h	hour
OECD	Organisation for Economic Cooperation and Development