Ethanol

Evaluation of the effects on reproduction, recommendation for classification

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp	:	aanbieding advies
Uw kenmerk	:	DGV/BMO-U-932542
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Bij brief van 3 december 1993, nr. DGV/BMO-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 repro-tox-lijst. Op deze lijst komen stoffen die volgens de richtlijnen van de Europese Unie ingedeeld moeten worden in categorie 1 of 2 wat betreft effecten op de voortplanting. De Gezondheidsraad is verzocht om voor stoffen een classificatie volgens de EU-criteria voor te stellen.

In 1996 heb ik hiervoor de Commissie Reproductietoxische stoffen ingesteld.

Hierbij bied ik u - gehoord de Beraadsgroep Gezondheid en Omgeving - een publikatie van de commissie aan over ethanol (alcohol). Deze publikatie is heden ter kennisname aan de Minister van Volksgezondheid Welzijn en Sport en aan de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer gestuurd.

Hoogachtend,
w.g.
prof. dr JJ Sixma

Ethanol

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. 2000/01OSH, The Hague, 19 April 2000

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie van reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie ethanol onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit, adviseert de commissie ethanol in categorie 1 *(Stoffen waarvan bekend is dat zij bij de mens de vruchtbaarheid schaden)* te classificeren en met R60 *(kan de vruchtbaarheid schaden)* te kenmerken.
- Voor ontwikkelingsstoornissen, adviseert de commissie om ethanol in categorie 1 *(Stoffen waarvan bekend is dat zij bij de mens ontwikkelingsstoornissen veroorzaken)* te classificeren en met R61 *(kan het ongeboren kind schaden)* te kenmerken.
- Voor effecten tijdens lactatie, adviseert de commissie om ethanol tevens met R64 *(kan schadelijk zijn via de borstvoeding)* te kenmerken.

Executive summary

On request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The Health Council's Committee for Compounds Toxic to Reproduction recommends to classify compounds toxic to reproduction according to the Directive 93/21/EEC of the European Union. In the present report the committee has reviewed ethanol.

The committee's recommendations are:

- For effects on fertility, the committee recommends that ethanol should be classified in category 1 *(Substances known to impair fertility in humans)* and labelled with R60 (*may impair fertility)*.
- For developmental toxicity, the committee recommends to classify ethanol in category 1 *(Substances known to cause developmental toxicity in humans)* and labelled with R61 *(may cause harm to the unborn child)*.
- For effects during lactation, the committee recommends that ethanol should be labelled with R64 *(may cause harm to breastfed babies)*.

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 according to the guidelines of the European Union (Directive 93/21/EEC) by the Health Council's Committee for Compounds Toxic to Reproduction. 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 and 2) of the European Union.

1.2 Committee and procedure

The present document contains the classification of ethanol 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 Mrs ir IDH Waalkens-Berendsen at the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research Institute, 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 April 1999, 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 in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

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

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

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up 1995. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection

* Organisation for Economic Cooperation and Development

of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1998. The results of this search were no reason for the committee to adjust the recommendations.

The committee chose to describe human studies in the text, starting with review articles. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

1.6 Presentation of conclusions

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

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

1.7 Final remark

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

* for definitions see Tox95

Chapter 2

Ethanol

2.1 Introduction

Name	:	Ethanol
CAS-no	:	64-17-5
Use	:	stimulant, desinfectant, solvent
Synonym	:	alcohol
Mol weight	:	46
Chem formula	:	C_2H_5OH

2.2 Human studies

Fertility studies

Chopra *et al* (Cho73) studied 13 male patients, 35 to 59 years of age, who had typically clinical features of chronic hepatic cirrhosis after chronic alcohol abuse. Six patients had gynecomastia and 10 out of 12 patients had atrophic testis; The testis of one patient could not be palpated because of massive ascites and scrotal oedema. The serum oestradiol-17ß was elevated in 8 out of 13 patients. Serum luteinizing hormone (LH) was above the normal range in 7 out of 13 patients.

Galvào-Teles *et al* (Gal73) studied 25 men with chronic liver disease; 8 men were chronic alcoholics. The plasma levels of 17ß-hydroxy-androgens, were significantly lo-

wer in the males with liver disease than in controls; this fall was most striking in the patients with alcoholic cirrhosis. Serum LH levels were increased in males with liver disease. Decreased libido, gynecomastia and small testis were found in 5, 4 and 4 out of 8 alcoholics, respectively. These clinical features were found in higher frequency in males with alcoholic cirrhosis; the authors presumed that this may be due to the greater reduction in unbound plasma androgens. In this study non-age-matched normal young men were used as controls.

Kent *et al* (Ken73) studied 22 men, 39 to 67 years of age, with a history of chronic alcoholism and hepatic insufficiency. Serum oestradiol, testosterone (T), follicle-stimulating hormone (FSH) and LH were measured. Only levels of testosterone were significantly different from those obtained in normal subjects. Eight males showed unilateral or bilateral small testis. Fifteen males reported a loss of libido.

Van Thiel *et al* (Thi78a) studied the effects of alcohol use on the hypothalamic-pituitary axis in 22 chronic alcoholic men aged 21-64 years and 14 healthy non-alcoholic men aged 18-35 years. Gynecomastia, testicular atrophy, impotence and reduced libido were found in 11, 12, 16 and 17 of the 22 alcoholic men, respectively. FSH, LH and TSH levels were increased in alcoholic men, whereas testosterone (T), triiodothyronine (T_3) and thyroxine (T_4) concentrations were decreased, when compared to the normal controls.

Pajarinen *et al* (Paj94) studied the effects of alcohol on spermatogenesis in a prospective autopsy study in 32 controls aged 55-61 years and 44 heavy drinkers aged 50-55 years; users of tranquilizers and diuretics were excluded. Sudden cardiac death, respiratory infections and other diseases were the primary cause of death. Spermatogenic arrest was found in 19 and 52 % of the controls and heavy drinkers, respectively. Five heavy drinkers had Sertolicell-only (SCO) syndrome. The mean testicular weight of the heavy drinkers was slightly lower than of the controls.

Spermatogenesis and testicular morphology of 195 men were studied; the men were categorized in a control group (<10 g alcohol per day, n=48) and 4 consumption groups (group I: 10-40 g alcohol per day, n=42; group II: 40-80 g alcohol per day, n=31; group III: 80-160 g alcohol per day, n=35 and group IV: >160 g alcohol per day, n=39) (Paj96). The groups had a slight difference in age; age of the control men was 55-60 years and of the highest dose group 48-54 years. Partial spermatogenic arrest was found in 33, 36, 42, 54 and 64 % of the control and consumption groups, respectively. SCO was detected in 1, 3 and 3 men of the groups with the highest alcohol consumption (group II, III and IV, respectively). A reduction was found in testicular weight for the two groups (III and IV) with the highest alcohol use.

Vilalta *et al* (Vil97) studied the effect of ethanol in 38 chronic alcoholics without chronic liver disease (reported ethanol consumption of about 200 g alcohol per day) and 19 age-matched controls. In alcoholics a significant increase in LH and FSH hormone

and a decrease in free androgen index* were observed. Seminal studies indicate sperm counts and motility were decreased in alcoholics whereas morphological abnormalities were increased when compared to controls.

An increase in dysmenorrhea, heavy menstrual flow and premenstrual discomfort was found by Wilsnack *et al* in a survey in 2552 women with increasing number of alcoholic drinks per day (Wil84). Becker *et al* studied the menstrual pattern, gynaecological disorders and infertility in 51 chronic alcoholic women and 51 controls and found that the alcoholic women were more prone to menstrual abnormalities and were at greater risk of gynaecological interventions, while they did not seem to have reduced fertility (Bec89).

Grodstein *et al* (Gro94) interviewed 1050 women from 7 infertility clinics and 3833 women who recently gave birth; they found an increase in infertility with alcohol use, due to disturbed ovulation or to endometriosis.

Remark

Alcohol induced sexual disfunction was initially attributed to liver disease (Cho73; Gal73; Ken73). However, additional studies were performed and it became clear that alcohol itself was the principle cause of the effects on fertility (Ban87; Thi81). Alcohol can induce testicular failure in the absence of alcohol induced liver disease.

Developmental toxicity

Alcohol-dependent women have been reported to give birth to children with a combination of anomalies, which is known as the foetal alcohol syndrome (FAS) (Lem68, Jon73a,b and Ros80). FAS is characterized by a spectrum of clinical features including prenatal and postnatal growth deficiency, morphological anomalies including distinctive facial appearance, skeletal abnormalities, cardiac defects, central nervous system dysfunction, including cognitive disabilities (Jon86, Gor90, Fro92 and Rob94).

FAS occurred in children of women with clearly identified drinking habits (Sok92). In some studies, the prevalence of FAS among alcohol-dependent women was as high as 26% (Sei78), and in others as low as 2% (Sok80). In an extensive review, Meyer described the behaviourial teratology of alcohol and concluded that there is considerable evidence for identifying alcohol as a behavioural teratogen in humans and animals (Mey86). More recently, Jacobson *et al* and Streissguth *et al* described neurobehaviourial effects of prenatal alcohol exposure (Jac93a,b and Stre94).

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* free androgen index = (total testosterone/ total sex steroid hormone binding globulin)*100

Lactation

Binkiewicz *et al* (Bin78) reported a breast-fed infant with increased levels of cortisol in the blood and a clinical pattern which closely resembled Cushing syndrome. During pregnancy the mother refrained from alcohol consumption. However, after birth she resumed drinking “in order to promote milk production”. In a random sample of breast milk 1 g alcohol/l was detected.

Lawton (Law85) analysed samples of breast milk and blood at fixed intervals after the ingestion of alcohol by 8 nursing mother. The results showed that alcohol appeared quickly in both fore- and hind-milk at a level equivalent to or higher than the corresponding blood samples.

Little *et al* (Lit89) studied in 400 infants the relation of the mother’s use of alcohol during breast feeding to the infant’s development at one year of age (299 infants from mothers with an absolute daily alcohol consumption of less than 1/2 oz. ethanol and 101 infants from mothers consuming more than 1/2 oz.). They found that ethanol ingested through breast milk had a slight but significant detrimental effect on motor development, but not on mental development in breast-fed infants.

Mennella *et al* (Men91) studied the effects of short-term alcohol consumption on flavour and odour of breast-milk and the feeding behaviour of their infants. Short-term alcohol consumption significantly and consistently increased the perceived intensity of the odour of human milk by a panel of adults. The infants sucked more frequently during the first minutes of feeding in cases where their mothers had consumed alcohol, but they consumed significantly less milk during the testing sessions in which their mothers drank alcoholic beverages.

Mennella and Gerrish (Men98) showed that short-term exposure to small amounts of alcohol in breast milk produced distinctive changes in the infant’s sleep-wake patterning.

2.3 Conclusion

Ethanol consumed in the form of alcoholic beverages affected human male fertility as concluded from effects as testicular atrophy, decreased libido and testosterone levels, and increased oestrogen levels. In addition, human female fertility was affected, as concluded from disturbances in the menstrual cycle.

Therefore, based on the human data [illegible] effects on the male reproductive system, the committee recommends [illegible] category 1 (substances known to impair fertility in humans) and [illegible] nd with R60 (may impair fertility).

Ethanol causes multiple congenital anomalies in humans (retarded growth, dysfunction central nervous system, external malformations), when exposure occurs during pre-

natal development. The incidence, nature and extent of these anomalies are dependent amongst others on time, duration and level of exposure during pregnancy.

The committee is of the opinion that a causal relationship exists between human exposure to ethanol and developmental effects and therefore recommends to classify ethanol in category 1 (substances known to cause developmental toxicity in humans) and to label the compound with R61 (may cause harm to the unborn child). This is irrespective the maternal (toxic) effects (see additional considerations, paragraph 1.3).

Ethanol consumed during lactation is excreted in breast milk and affects infant's sleep-wake patterning, motor development and can cause pseudo-Cushing syndrome. Therefore, the committee recommends to label ethanol with R64 (May cause harm to breastfed babies) as well.

Proposed classification for effects on fertility

Category 1, R 60.

Proposed classification for developmental toxicity

Category 1, R 61.

Proposed labelling for effects during lactation

R 64.

For the committee,
The Hague, 19 April 2000

dr ASAM van der Burght, scientific secretary	dr BJ Blaauboer, chairman

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Annexes

Annex **A**

The committee

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  Toxicologist; Research Institute of Toxicology, Utrecht
- JN van den Anker
  Professor of pediatrics and Neonatology; Erasmus University, Rotterdam
- AM Bongers, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- HFP Joosten
  Toxicologist; NV Organon, Department of Toxicology and Drug Disposition, Oss
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- ASAM van der Burght, *scientific secretary*
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The first draft of the present document was prepared by IDH Waalkens-Berendsen, from the TNO Nutrition and Food Research Institute in Zeist, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: E Vandenbussche-Parméus.
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 1999 for public review. The following persons and organisations have commented on the draft document:

- L Appelman, C Braun, E Houthoff
  Akzo Nobel, Arnhem
- C Jeukenne,
  Ethyl Alcohol Group, Bruxelles, Belgium
- JR Kelsey
  BP Chemicals Limited, Hull, United Kingdom
- H Lindemann
  Bayer AG, Wuppertal, Germany
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- EC Rietveld
  Diosinth bv, Akzo Nobel, Oss
- CJ Halm
  FME-CWM, Zoetermeer

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 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 resuts 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 in-

terfere 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, peri-postnatal defects, and impaired postnatal mental 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 exposure is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

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

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

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**

Abbreviations

Abbreviations used:

- body weight
- day
- female(s)
- intraperitoneal
- intravenous
- male(s)
- number
- no observed adverse effect level
- Organisation for Economic Cooperation and Development
- postnatal
- week