Selenium and its compounds

Evaluation of the effects on reproduction, recommendation for classification

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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

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Contents

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 voor reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie seleen en seleenverbindingen onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit meent de commissie dat er onvoldoende gegevens beschikbaar zijn. Zij adviseert daarom seleen en seleenverbindingen niet te classificeren.
- Voor ontwikkelingsstoornissen, meent de commissie dat er voldoende gegevens zijn die laten zien dat deze verbindingen de ontwikkeling van het nageslacht niet schaden. Zij adviseert om seleen en seleenverbindingen niet te classificeren.
- Voor effecten tijdens de lactatie, adviseert de commissie om seleen en seleenverbindingen 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 a classification for compounds toxic to reproduction according to the Directive 93/21/EEC of the European Union. In the present report the committee has reviewed selenium and its compounds.

The committee's recommendations are:

- For effects on fertility, the committee recommends no classification of selenium and its compounds due to a lack of appropriate data.
- For developmental toxicity, the committee is of the opinion that sufficient data show that no classification of selenium and its compounds is indicated.
- For effects during lactation, the committee recommends that selenium and its compound 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 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 and 2) of the European Union.

1.2 Committee and procedure

The present document contains the classification of selenium and its compounds 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 AE Smits-van Prooije and Mrs ir DH 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, 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, 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.
- 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).
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- 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.
- 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 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 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.

Animal data are described in the text and summarised in Annex D.

* Organisation for Economic Cooperation and Development

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 preclude 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* 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 organisations.

* for definitions see Tox95

Chapter 2

Selenium and its coumpounds

2.1 Introduction

Name	:	Selenium
Chemical formula	:	Se
Atomic weight	:	78.96
Use	:	as decolourizer in glass and ceramics industry, for rectifiers, photocells, solar batteries, television cameras, xerography in the electronics industry, as additive to medicated shampoos and dietary supplement

Selenium is an essential trace element. It has an important metabolic role as a co-factor of the enzyme glutathione peroxidase which is, together with vitamin E, catalase and superoxide dismutase, a component of one of the antioxidant defence systems in the body. Limited data are available on the effects of a selenium deficiency in humans. A deficiency might result in Keshan disease, a selenium responsive endemic cardiomyopathy that mainly affects children and women of child-bearing age (Yan88).

In 1989, the Netherlands Food and Nutrition Council recommended a dietary selenium intake of 50-150 µg per day for male and female adults (Voe89).

2.2 Human studies

Fertility

Bleau *et al.* studied semen samples of 125 men of couples consulting for infertility. Relevant information of 101 couples about their reproductive performance was collected in a follow-up study 4.5-5 years after the initial study. In men, a positive correlation was observed between sperm count and semen Se concentration. Sperm motility was maximal at semen Se levels between 50 and 69 ng/ml; below and above this range the motility was lower and the incidence of asthenozoospermia was higher. Low semen Se levels were associated with a low pregnancy rate. A high semen Se level was associated with a high abortion rate and female reproductive failures (both partners are usually exposed to the same diet and environment). Exposure data to Se was not reported (Ble84).

Takasaki *et al.* studied semen samples of 32 healthy fertile and 73 infertile males. The following conclusions could be drawn from this study: 1. Se concentrations in whole semen and seminal plasma were not significantly different between the fertile group and the infertile group; 2. Sperm Se concentrations were significantly higher in the infertile group; 3. A negative correlation was observed between sperm Se levels and sperm motility; 4. The results suggests that an increased Se level in sperm was related to male infertility, especially with regard to deterioration of sperm motility and spermatogenesis (Tak87).

Roy *et al.* measured the Se concentration in seminal plasma of 211 healthy men attending a male fertility clinic and observed no significant correlation between the Se levels in the seminal plasma and sperm count or motility (Roy90).

From the studies of Bleau (1984), Takasaki (1987) and Roy (1990) no clear correlation between seminal Se concentration and effects on male fertility could be detected (Ble84; Tak87; Roy90). In the study of Takasaki (1987), a negative correlation between male fertility and sperm Se concentration was observed. The exposure to Se was not determined in these studies; The committee is of the opinion that the changes in Se concentrations (in semen or seminal plasma) might be a result of endogenous factors (metabolism/kinetics) and not a result of exposure to selenium. Therefore, the results of these studies were therefore considered insufficient evidence for classification of selenium for fertility.

Developmental toxicity

A five-year study with 8 women occupationally involved with selenium reported 5 pregnancies ending in abortion and one that resulted in a full-term baby with bilateral club-foot. Comparison between urinary selenium levels of the exposed group and a non-exposed group did not show a correlation between selenium levels and the abortions (Rob70).

An epidemiological study was performed in a highly seleniferous area in rural China. Loss of hair and nails, lesions of skin and abnormalities of the nervous system were found; blood selenium levels averaged 3.2 µg/ml (range 1.3-7.5 µg/ml), compared with a blood level of 0.095 µg/ml in a normal selenium area: the breast-fed children in this affected area remained unaffected (Yan83).

A study on the effects during pregnancy of among others selenium in drinking water covered a one-year period in 1980-1981 and included 270 children with a severe congenital heart disease and 665 controls. The detection limit of selenium was 0.1 µg/l and the highest observed level was 10 µg/l. A weak inverse relationship between exposure to selenium and incidence of heart diseases was observed (Zie88).

Selenium in human breast milk

The primary factor determining the concentration of Se in human milk is maternal Se intake (Ala95), although Se concentration in human milk can be affected by several other factors such as time postpartum. Colostrum was found to contain higher Se levels than mature milk (Ben95, Tam95, Kim98); absolute intake of Se of breast-fed children increased with day post partum due to increased milk intake. A safe and adequate range for Se intake was found to be 10-40 µg/day (Sub89) for infants of 0-6 months.

Mature human milk can exhibit a wide variation in Se content due to geographic location (She75, Kum83, Mos88, Yan89a and b, Brä91, Arn93, Ben95, Brä97). In seleniferous (high Se) areas the Se content of the local food (Yan89a) is increased; plant food accounts for more than 90% of the dietary intake of Se. The daily Se intake of infants within the first year of age were 11.8, 24.3 and 94.6 µg/day in low, medium and high-Se areas in China, respectively, as calculated from daily breast milk consumption (Yan89a). An increase in the incidence of mottled enamel teeth was described with increase in Se level (Yan89b). In contrast to the studies of Hadjimarkos *et al*. 1952 (Had52) among school children in Oregon and the Montana-Oregon-South Dakota study in high-Se areas (Lud69) who detected increased levels of caries, in the studies of Yang *et al*. 1989 (Yan89b) in the Enshi county a decrease in dental caries was observed with increasing concentrations of Se. In the Enshi county increased fluoride (F) levels of the soil were usually associated with the increased Se levels. In the Wyoming study of Tank

and Storvick 1960 (Tan60) the prevalence of caries among school children in areas that were high in both Se and F was 41% greater compared with that of subjects living in areas high in F but low in Se.

In a study on the health effects of high dietary maternal intake, breast milk blood and toe-nails were collected from 143 women (20-24 day of lactation) living in the Venezuelan states Yaracuy and Portuguesa (Brä97). The regions differed in Se content. The Se concentration in breast milk was found to be proportional to dietary intake. A significant inverse relation was detected between Se and zinc (Zn) in breast milk. Anthropometric examinations of the seleniferous regions showed that the mean height of children (2 and 7 years) differed significantly from the height in Caracas at all socio-economic levels for both sexes and all ages (Brä91). The reported difference in the rate of growth might be associated with the lowered Zn concentration and the consequent reduced biological availability of zinc. However, the authors did not exclude other factors.

2.3 Animal studies

Tables 1, 2.1 and 2.2 summarize the fertility and developmental toxicity studies with selenium in experimental animals.

Fertility

Two groups of 12 male Wistar rats were given a diet consisting of normal wheat or wheat grown in selenium-rich soil (containing ± 12.5 ppm Se) for 4 weeks. Food consumption in the exposed group was significantly reduced. Upon sacrifice, body weight and testis weight were significantly reduced. Histological examination revealed oedema of the testicular interstitial tissue, necrosis of the spermatogenic cells and degeneration of the spermatids (Par89).

Five groups of 2-4 female monkeys (Macaca fascicularis) were daily gavaged for 30 days with 0, 25, 150, 300 or 600 µg L-selenomethionine/kg body weight, starting 5 days after the onset of menstruation. After 10 days of treatment, 2 females from the 600 µg/kg group died of toxic effects. All dose groups showed treatment-related clinical signs such as anorexia, dermal lesions, gastrointestinal distress, hypothermia. Animals of all dose groups lost weight during treatment, but a severe increase in weight loss was observed in the 300 µg group, in combination with disturbances of the menstrual cycle, reduced serum progesterone, luteal phase length, urinary oestrogen excretion and increased intermenstrual intervals (Cuc89).

Developmental toxicity

Büttner (1963) administered sodium selenite in drinking water (2.3 and 4.6 ppm Se) of pregnant rats and their pups until postnatal day 120 (Büt63). Administration of both levels reduced the number of pups born and also decreased the weight gain as a result of reduced food intake (general toxicity). Se-treated pups developed more carious lesions than controls; the carious lesions was also more extensive.

Groups of pregnant New Zealand White rabbits were exposed by intravenous injection to 0 or 1.75 mg/kg body weight (bw) sodium selenite on day 9 of gestation. Two of 14 treated does died; 5 does produced a live litter. Postimplantation loss was significantly increased. No malformations were observed (Ber77).

Groups of 23 pregnant CD-1 mice were daily gavaged with 0 or 10 mg sodium selenite/kg bw from gestation day 8-12. Significant reduction in maternal body weight gain and number of live pups on postnatal day 1 and 3 were observed; pup weights were normal (Che82).

Groups of 6-11 pregnant IVCS mice were injected subcutaneously with 0, 1, 1.5, 2.1, 3.2, 4.6 or 6.8 mg Se/kg bw (= 0, 12.7, 18.6, 27.2, 40.0, 58.8 or 86.3 µmol Se/kg bw, respectively) on gestation day 12 or 16. On day 12 and day 16, all 6.8 mg/kg bw treated females died; On day 12, most of the 4.6 mg/kg bw group died (the rest aborted), and on day 16 all of the 4.6 mg/kg bw treated females died. On day 16, the 3.2 mg/kg bw treated females died or aborted; on day 12, the 3.2 mg/kg bw treated females had a statistically significantly reduced body weight; the number of implantation sites and live born was statistically significantly reduced. On day 12, the 1.5 and 2.1 mg/kg bw treated females resulted in a non-significant reduction in maternal body weight, number of implants, number of live born pups and birth weight. On day 16, part of the 2.1 mg/kg bw treated females aborted, and the remainder delivered litters with nearly normal size and birth weights. On day 12, treatment with 1 mg Se/kg bw resulted in a significant increase in the number of liveborn pups and implantation sites. It was concluded that pregnant dams are more susceptible on gestation day 16 than on day 12 and that dose-dependent foetotoxic effects are more apparent on day 12 (Yon83).

Groups of 5-9 pregnant CD-1 mice were injected intravenously with 0, 1.3, 1.6, 2.0 or 2.5 mg Se/kg bw (= 0, 16.4, 20.5, 25.6 or 32.0 µmol Se/kg bw, respectively) as either selenodiglutathione or sodium selenite on gestation day 12. All females from the high dose selenodiglutathione and most females from the high dose sodium selenite died. Maternal body weight gain was reduced in both treatment groups, at all dose levels. Resorptions were not observed; stillbirths were found in 1.6 mg/kg bw and 2.0 mg/kg bw selenodiglutathione and 2.0 m/kg bw sodium selenite groups. Numbers of implantation sites and live born pups were similar in the treatment groups and slightly

but not significantly reduced when compared to the controls; birth weights were reduced in all dose groups (Yon84).

Groups of 18 pregnant Bom: NMRI mice were exposed to approximately 0, 0.2, 2.0 or 10 mg sodium selenite/kg bw via the drinking water from gestation day 11 to 18. About two-third of the high dose females aborted, and in the remaining litters foetal body weight was significantly reduced. The lower dose levels did not significantly affect foetal or placental weight. No malformations were reported (Hau87).

Groups of 22 pregnant CD-1 mice were daily gavaged with 0 or 7 mg sodium selenite/kg bw from gestation day 7-14. The reproduction index (number females with live litter/number pregnant females) was slightly but not significantly reduced; no other effects on females or offspring were observed (Pla85).

Groups of 50 pregnant CD-1 mice were daily gavaged with 0, 3.5, 5.0, 7.0 or 14.0 mg sodium selenite/kg bw from gestation day 6-13. Exposure up to 7 mg/kg bw had no adverse effects; 14 mg/kg bw resulted in a significant increase of maternal mortality, decrease in weight gain, a reduction in viable litters, birth weight, postnatal weight gain and pup viability. The number of pups born per viable litter was normal (Har87).

Groups of 22 pregnant Sprague-Dawley rats were daily gavaged with 0, 1.5 or 3.0 mg Se/kg bw from gestation day 7-16. Eleven females were sacrificed at gestation day 22; the others were allowed to litter and the general health and development of the offspring were followed. In the teratology study a dose-dependent decrease in maternal body weight and in food consumption was observed. Mean number of corpora lutea and implantation sites was normal; the number of resorptions increased slightly but not significantly in a dose-dependent way. Foetal weights decreased in a dose-dependent way, statistically significant in the high dose group. The number of external and visceral anomalies was low; skeletal anomalies showed a dose-related increase, among them partially unossified skull bone, bipartite and partly unossified sternebrae. In a similar teratology study, using 7.5 and 15 mg Se/kg bw from gestation day 7 - 9/12, maternal mortality was observed in the high dose group, and in the remaining litters anophthalmia and exencephaly. The litters allowed to be delivered showed a slight but persistent dose-dependent decrease in body weight, but no differences were observed in development or behaviour (Dan88).

Groups of 4-9 pregnant Syrian hamsters were treated with doses ranging from 1.8 - 8.7 mg (23 - 220 µmol) Se/kg bw for sodium selenite, from 7.0 - 8.7 (90 - 110 µmol) mg Se/kg bw for sodium selenate and from 5.9 - 7.9 mg (75 - 100 µmol) Se/kg bw for L-selenomethionine either via gavage, or intravenous injection or osmotic minipump infusion. Administration occurred at gestation day 8 or day 5-8. Additional groups received by gavage a total dose of either 5.9 mg (75 µmol) or 7.9 mg (100 µmol) Se/kg bw as selenomethionine, divided over gestation day 5-8. The higher doses resulted in reduced food consumption and maternal death (for selenite and selenate). All

dose-groups displayed inanition and marked loss of body weight. Foetal body weight and crown-rump length were decreased in the higher dose groups. Malformations (encephalocele and exencephaly) were observed only at or slightly below levels that produced maternal toxicity. I.v. injection caused effects at lower levels than oral administration; a single dose of selenomethionine caused more effect than constant infusion of the same dose (Fer90).

Groups of 7-9 pregnant monkeys (*Macaca fascicularis*) were daily gavaged with 0, 25, 150 or 300 µg L-selenomethionine/kg bw from gestation day 20-50. Four to 7 per group were sacrificed on gestation day 100, 2-3 were allowed to deliver. Dose dependent maternal toxicity resulted in the mid and high dose groups in reduced food consumption, emesis, significant reduction in body weight and a low incidence of pregnancy loss. High dose foetal weights and measures were slightly but not significantly reduced. No malformations were observed. Postnatal growth and development were normal (Tar91).

2.4 Overall conclusion

From the studies of Bleau (1984), Takasaki (1987) and Roy (1990) no clear correlation between seminal Se concentration and human male fertility could be detected (Ble84, Tak87 and Roy90). Takasaki *et al* (1987) observed a negative correlation between male fertility and sperm Se concentration. The committee is of the opinion that endogenous factors (metabolism/kinetics) as a cause of the changed Se-levels cannot be excluded, because Se exposure levels were not measured. These studies were therefore considered insufficient evidence for classification of selenium for fertility.

No relationship was reported between selenium-exposed women and children with congenital malformations (Rob70; Yan83; Zie88).

Therefore, from the human data the committee cannot draw definite conclusions concerning the effects on human fertility or developmental toxicity data after exposure to selenium and its compounds.

The impact of exposure to Se in animal experiments depended somewhat on the age at which administration took place, the route of administration and the biochemical form used to administer Se. The effects found in different species tested were fairly consistent.

Exposure to Se in experimental animals is among others accompanied by loss of weight, both in parental animals and in (foetal) offspring.

Adverse effects on fertility, among them histological lesions of testes (Par89) and disturbances of the female cycle, were observed at levels of overt toxicity in only one study (Cuc89). Therefore, in view of the animal data, the lack of appropriate data preclude assessment of the compound for fertility.

Administration of Se during pregnancy to animals at maternotoxic levels mostly resulted in an increased incidence of resorptions, abortions or stillbirths (Ber77; Che82; Dan88; Hau87; Har87; Yon83; Yon84). A low incidence of congenital anomalies and malformations was reported, among them anophthalmia, exencephaly and encephalocele, but only at levels causing overt toxicity (Dan88; Fer90). In animal teratogenicity studies most effects were observed at levels causing overt toxicity. Exposure below these levels hardly caused effects. In view of the animal data on developmental toxicity, selenium is not classified as a substance which causes concern for humans.

Dietary intake of Se during lactation affects the concentration of Se in human breast milk. A significant inverse relation was detected between Se and Zn in breast milk (Brä97). Zn deficiency was assumed to lead to retarded growth (GR98). In animal and human epidemiological studies an increased Se intake was associated with dental caries (But63; Had52; Had61; Tan60; Lud69)

Although the committee recommends not to classify and subsequently label selenium for effects on development (developmental effects were observed at or near dosages which cause general toxicity), the committee proposes to label selenium with R64 (may cause harm for breastfed babies).

Proposed classification for fertility

Lack of appropriate data preclude assessment of the compound for fertility.

Proposed classification for developmental toxicity

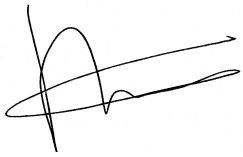
Sufficient data show that no classification for developmental toxicity is indicated.

Proposed labelling for effects during lactation

R64.

For the committee,
The Hague, 25 August 1999

dr ASAM van der Burght,
scientific secretary



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Annexes

Annex **A**

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Annex **B**

Comments on the public draft

A draft of the present report was released for public review in April 1999. No organisations or persons commented on the draft document.

Annex **C**

Guideline (93/21/EEG) 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 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 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

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 Fertility studies with selenium.

authors	species	route	experimental period	dose	findings	remarks
Parshad and Sud (1989)	Wistar rat (males)	diet	administration: 4 w	0, ± 12.5 mg Se/kg feed (or 0.625 mg/kg bw)	12.5 mg/kg feed: sign. reduction food consumption, body and testis weight; testicular interstitial oedema, spermatogenic cell necrosis, degeneration spermatids	Se level control diet not given
Cuckierski *et al*. (1989)	Macaque monkey	gavage	administration: 30 d starting from d 5 after menstruation	0, 25, 150, 300, 600 µg Se/kg bw	600 µg: death of 2 animals all dose groups: anorexia, dermal lesions, gastrointestinal distress, hypothermia, reduction bw (stat. sign.in 300 µg group) 300 µg: disturbance menstrual cycle, reduced serum progesteron, luteal phase length, oestrogen excretion; increase intermenstrual interval	

d = day; stat.sign. = statistically significant; w = week

Table 2.1 Developmental toxicity studies with selenium .

authors	species	route	experimental period	dose	findings	remarks
Büttner (1962)	Wistar rats (female)	drinking water	gestation and lactation period and offspring for 120 days	0, 5 and 10 ppm sodium selenite	5 and 10 ppm increased mean number and extend of carious lesions	decreased fertility and weight gain
Berschneider *et al*. (1977)	New Zealand White rabbit	i.v. injection	administration: gestation d 9	0, 1.75 mg sodium selenite/kg bw	1.75 mg: 14% maternal death; 7.2% delivered a live litter; sign. increase postimplantation loss; no malformations	
Chernoff and Kavlock (1982)	CD-1 mouse	gavage	administration: gestation d 8-12	0, 10 mg sodium selenite/kg bw	10 mg: sign. reduction maternal body weight gain, no. live pups postnatal d 1 and 3. Pup weights normal	
Yonemoto *et al*. (1983)	IVCS mouse	s.c. injection	administration: gestation d 12 or 16	0, 1, 1.5, 2.1, 3.2, 4.6, 6.8 mg Se/kg bw	6.8 and 4.6 mg/kg: all females died or aborted 3.2 mg/kg: maternal death, abortions (d16); sign. decreased resorptions, live born pups, reduction birth weight (NS), decreased maternal bw (NS) (d12) 2.1 mg/kg: abortions, decreased no. liveborn pups (d16); 2.1 and 1.5 mg/kg: reduction maternal bw (NS) 1 mg/kg: sign. increased no. live born pups, no. implants (d12)	
Yonemoto *et al*. (1984)	CD-1 mouse	i.v. injection	administration: gestation d 12	0, 1.3, 1.6, 2.0, 2.5 mg Se/kg bw	2.5 mg/kg: maternal death all levels: reduction maternal weight gain, birth weights, no. implantation sites (NS), live born pups (NS). No resorptions	injected was either sodium selenite or selenoglutathione
Plasterer *et al*. (1985)	CD-1 mouse	gavage	administration: gestation d 7-14	0, 7 mg sodium selenite/kg bw	7 mg/kg: not-sign. decreased reproduction index; no apparent effects on maternal toxicity	
Hau *et al*. (1987)	Bom: NMRI mouse	drinking water	administration: gestation d 11-18	0, 0.2, 2.0, 10.0 mg sodium selenite/kg bw	10 mg: 66% abortion; sign. reduction foetal body weight 0.2-2 mg: no effects foetal or placental weight; No malformations	

d=day; i.v.= intravenous; s.c.= subcutaneous; NS= not significant

Table 2.2 Developmental toxicity studies with selenium.

authors	species	route	experimental period	dose	findings	remarks
Hardin *et al*. (1987)	CD-1 mouse	gavage	administration: gestation d 6-13	0, 3.5, 5.0, 7.0, 14.0 mg sodium selenite/kg bw	14 mg: sign. increased maternal death, decreased weight gain, reduction viable litters, birth weight, postnatal weight gain, pup viability. 3.5-7 mg: no adverse effects	
Danielson *et al*. (1988)	Sprague-Dawley rat	gavage	administration: gestation d 7-16 sacrifice (½ of the females): gestation d 22	0, 1.5, 3.0 mg Se/kg bw	Dose-dependent decreased maternal body weight, food consumption; not-sign. increased resorptions, skeletal anomalies. 3 mg: sign. decreased foetal weight - dose dependent not-sign. persistent reduction in pup body weight; no effects development, behaviour	
idem	Sprague-Dawley rat	gavage	administration: gestation d 7 - 9 or 12	7.5, 15 mg Se/kg bw	15 mg: maternal death, anophthalmia, exencephaly	
Ferm *et al*. (1990)	Syrian hamster	gavage, i.v. injection, osmotic minipump infusion	administration: gestation d 8 or d 5-8 sacrifice: gestation d 13	1.9-19.0 mg Se/kg bw (sodium selenite) 4.35-20.8 mg Se/kg bw (sodium selenite) bw 14.7-49.4 mg Se/kg bw (selenomethionine)	higher doses selenite/selenate: maternal death, reduction food consumption, encephalocele and exencephaly all dose groups: marked reduction body weight, inanition i.v.injection has more effect on lower doses than oral administration; a single dose Se-methionine caused more effect than extended infusion of the same dosis	
Tarantal *et al*. (1991)	Macaque monkey	gavage	administration: gestation d 20-50 sacrifice (± of the females): gestation d 100	0, 25, 150, 300 µg/kg bw	300 and 150 µg: emesis, reduction food consumption, body weight (sign.), pregnancy loss, not-sign. reduction foetal weight, measures. No malformations. Normal postnatal growth and development	

d = day; i.v. = intravenous; sign. = significant

Annex **E**

Abbreviations

Abbreviations used:

bw	=	body weight
d	=	day
F	=	female(s)
i.p.	=	intraperitoneal
i.v.	=	intravenous
M	=	male(s)
n	=	number
NOAEL	=	no adverse effect level
OECD	=	Organisation for Economic Cooperation and Development
PN	=	postnatal
s.c.	=	subcutaneous
w	=	week