Nickel and its compounds

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 'Nickel and its compounds'.
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U 97/AvdB/ra/543-L6
Bijlagen : 1
Datum : 18 februari 2003

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 nikkel en nikkelverbindingen. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. Ik wil u er op wijzen dat de commissie adviseert nikkelcarbonyl wat betreft de effecten op de ontwikkeling van het nageslacht in categorie 3 te classificeren. Dit omdat de specifieke effecten op de ontwikkeling van het nageslacht gevonden zijn in één studie en na een éénmalige blootstelling. Het advies van de commissie wijkt af van het standpunt van de Europese Commissie, die nickelcarbonyl wat betreft de effecten op de ontwikkeling van het nageslacht in categorie 2 heeft geclassificeerd. De redenen van de Europese Commissie heeft de commissie echter niet kunnen achterhalen. Dit verschil van inzicht heeft echter geen gevolgen voor het opnemen van nikkelcarbonyl op de hierboven genoemde lijst van voor de voortplanting vergiftige stoffen.

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

Hoogachtend,

prof. dr JA Knottnerus

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 7520
E-mail: A.vd.Burght@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23
www.gr.nl

Nickel and its compounds

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. 2003/05OSH, The Hague, February 18, 2003

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).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

Preferred citation:
Health Council of the Netherlands: Committee for Compounds toxic to reproduction. Nickel and its compounds; Evaluation of the effects on reproduction, recommendation for classification. The Hague: Health Council of the Netherlands, 2003; publication no. 2003/05OSH.

ISBN: 90-5549-466-6

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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 nikkel en nikkelverbindingen onder de loep genomen.

De aanbevelingen van de commissie zijn:

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

met R63 (*mogelijk gevaar voor beschadiging van het ongeboren kind*) te kenmerken
  - voor effecten tijdens de lactatie is de commissie van mening dat er onvoldoende gegevens zijn om nikkelcarbonyl te kenmerken.
- Oplosbare nikkelzouten.
  - voor effecten op de fertiliteit adviseert de commissie om de oplosbare nikkelzouten in categorie 3 te classificeren (*stoffen die in verband met hun mogelijke voor de vruchtbaarheid van de mens schadelijke effecten reden geven tot bezorgdheid)* en met R62 (*mogelijk gevaar voor verminderde vruchtbaarheid*) te kenmerken
  - voor effecten op de ontwikkeling adviseert de commissie de oplosbare nikkelzouten in categorie 2 te classificeren (*stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken*) en met R61 (*kan het ongeboren kind schaden*) te kenmerken
  - voor effecten tijdens lactatie adviseert de commissie om oplosbare nikkelzouten met R64 (*kan schadelijk zijn via de borstvoeding*) te kenmerken.
- Onoplosbare nikkelzouten
  - voor effecten op de fertiliteit adviseert de commissie om de onoplosbare nikkelzouten niet te classificeren wegens onvoldoende geschikte gegevens
  - voor effecten op de ontwikkeling adviseert de commissie om de onoplosbare nikkelzouten niet te classificeren wegens onvoldoende geschikte gegevens
  - voor effecten tijdens de lactatie is de commissie van mening dat er onvoldoende gegevens zijn om de onoplosbare nikkelzouten 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 nickel and its compounds.

The committee's recommendations are:

- Metallic nickel
  - for effects on fertility, the committee recommends no classification of metallic nickel, due to a lack of appropriate data
  - for developmental toxicity, the committee recommends not to classify metallic nickel due to a lack of appropriate data
  - the committee is of the opinion that a lack of appropriate data precludes the labelling of metallic nickel for effects during lactation.
- Nickel carbonyl
  - for effects on fertility, the committee recommends no classification of nickelcarbonyl, due to a lack of appropriate data
  - For effects on development, the committee recommends to classify nickelcarbonyl in category 3 (*substances which cause concern for humans owing to possible developmental effects*) and to label the compound with R63 (*possible risk of harm to the unborn child*).

- the committee is of the opinion that a lack of appropriate data precludes the labelling of nickelcarbonyl for effects during lactation.

- Soluble nickel salts
  - For effects on fertility, the committee recommends to classify the soluble nickel salts in category 3 (*substances which cause concern for human fertility*) and to label the compounds with R62 (*possible risk of impaired fertility*)
  - For effects on development the committee recommends to classify the soluble nickel salts in category 2 (*substances which should be regarded as if they impair fertility in humans*) and to label the compounds with R61 (*May cause harm to the unborn child)*.
  - for effects during lactation, the committee recommends that the soluble nickel salts should be labelled with R64 (*may cause harm to breastfed babies).*
- Insoluble nickel salts
  - for effects on fertility, the committee recommends no classification of the insoluble nickel salts, due to a lack of appropriate data
  - for developmental toxicity, the committee recommends not to classify the insoluble nickel salts due to a lack of appropriate data
  - the committee is of the opinion that a lack of appropriate data precludes the labelling of insoluble nickel salts 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 nickel 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 dr ir MEM Kuilman, ir DH Waalkens-Berendsen and dr ir APM Wolterbeek 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 2002, 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 breastmilk 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 labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceedence of the exposure limit for the general population, eg the acceptable daily intake (ADI).

* Organisation for Economic Cooperation and Development

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up to 2000. 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 well as several websites regarding (publications on) toxicology and health. References are divided in literature cited and literature consulted but not cited.

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.

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

Introduction

Nickel is a metallic element. Nickelcarbonate, nickel subsulfide and nickel oxide are insoluble in water, whereas nickel chloride, nickel sulphate, nickel acetate and nickel nitrate are water soluble. Nickel carbonyl is a volatile colourless liquid.

A fundamental difference exists between soluble and insoluble nickel compounds in their kinetic pattern and extracellular and intracellular bioavailability. The soluble nickel salts are rapidly cleared from the body and enter cells only to a limited degree. They become bioavailable only at higher dose levels and with continuous exposure. Insoluble nickel compounds such as nickel oxide and nickel subsulfide have a higher tendency to be retained at their site of application. They enter cells via active phagocytosis and achieve higher and long-lasting bioavailability. Particle size and surface charge are important factors for the induction of phagocytosis. Nickel carbonyl, a volatile liquid, readily traverses lipid membranes and is practically insoluble in water. Metallic nickel is insoluble in water.

Soluble nickel salts and insoluble nickel compounds are absorbed 10% or less from the gastrointestinal tract. Absorption via the lungs depends on particle size and solubility of the compound, ie nickel subsulfide and nickel oxide (low solubility) are retained in the lungs, whereas gaseous nickel carbonyl is readily absorbed from the lungs. After absorption, nickel carbonyl is progressively oxidized within erythrocytes and other cells to liberate Ni^{2+} which is excreted in the urine and carbon monoxide which is eliminated in the expired breath (Sun77). Absorption of soluble nickel salts after dermal application is indicated in rats.

Administration of nickel carbonyl or soluble nickel salts by respiratory or parenteral routes results in a wide distribution of nickel in the human body. In contrast, nickel is retained at the site of administration following parenteral dosage of insoluble nickel compounds or nickel dust (nickel oxide, nickel subsulfide). Ultimately a significant fraction of the nickel in substances taken up by inhalation, ingestion, implantation or injection is excreted as Ni^{2+}. Absorbed nickel accumulates in the kidneys, liver and lungs. Excretion is mainly via the urine. Nickel ions cross the placenta and can be present in maternal milk during lactation. Little is known about the kinetics of metallic nickel. Camner *et al*. (Cam84) exposed rabbits inhalatory to metallic nickel dust and observed alveolar macrophages, alveolar epithelial type II cells and phospholipids to be adversely affected. Identical effects were observed after exposure to nickel chloride.

Nickel carbonyl is the only nickel compound for which there is clearly identified acute toxicity in man. It exerts its effects primarily in the lungs and central nervous system and can cause death over a period of days from respiratory and cardiac failure. Pathological lesions have been reported in lungs, brain, liver, kidneys, adrenals and spleen of diseased workers.

Repeated inhalation of insoluble nickel results in lung inflammation and fibrosis. Nickelsubsulfide is at least 10 times more effective than nickel oxide in this respect. By the oral route insoluble nickel compounds have a low toxicity. Epidemiological studies of cancer mortality in nickel refinery workers indicate that increased risks of cancers of the nasal cavities and lungs have occurred primarily among workers exposed to nickel subsulfide and nickel oxide.

Inhalation or intratracheal administration of soluble nickel salts produces lung inflammation and bronchial hyperplasia. Soluble nickel salts are harmful by ingestion. The most prominent signs of intoxication are nausea, diarrhoea, giddiness and headache; in addition, ataxia was observed in animals. Liver and kidney damage have been observed after repeated administration of soluble nickel salts percutaneously or parenterally. High risks for lung and nasal cancers were observed among electrolysis and hydro metallurgy workers primarily exposed to nickel sulfate.

Although soluble nickel salts are primary skin and eye irritants at high concentrations, nickel sensitisation is of much greater importance. Nickel, as nickel salts or as the metal, is a very potent human skin sensitiser. Nickel also produces respiratory sensitisation reactions, particularly asthma. Repeated exposure to very dusty conditions in nickel mining and nickel refining has been reported to cause increased mortality from non-malignant respiratory disease or pneumonconiosis.

For the purpose of this report nickel and nickel compounds are categorised, in view of their kinetic and toxicological characteristics, as follows: metallic nickel, nickel carbonyl, soluble nickel salts and insoluble nickel compounds.

Chapter 3

Metallic Nickel

3.1 Properties

Name	:	nickel
CAS-no	:	7440-02-0
Use	:	steel production, hydrometallurgical nickel refining, barrier material production
Mol weight	:	58.69
Chem formula	:	Ni

3.2 Human studies

Fertility

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

Developmental studies

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

Lactation

See chapter 7.

3.3 Animal studies

Fertility

No animal studies were found regarding the effects of exposure to metallic nickel on fertility.

Developmental studies

No animal studies were found regarding the effects of exposure to metallic nickel on development.

Lactation

See chapter 7.

3.4 Conclusion

No studies were available concerning the effects of metallic nickel on fertility in man or animals. Therefore, the committee recommends not to classify metallic nickel with respect to effects on fertility because of a lack of appropriate data.

No studies were available concerning the developmental effects of metallic nickel in man or animals. Therefore, the committee recommends not to classify metallic nickel with respect to effects on development because of a lack of appropriate data.

Proposed classification for fertility

A lack of appropriate data precludes assessment of metallic nickel for fertility.

Proposed classification for developmental toxicity

A lack of appropriate data precludes assessment of metallic nickel for development.

Proposed labelling for effects during lactation

See chapter 7.

Chapter 4

Nickel carbonyl

4.1 Properties

Name	:	nickel carbonyl
CAS-no	:	13463-39-3
Use	:	refinery processes
Mol weight	:	170.73
Chem formula	:	$Ni(CO)_4$
LD_{50}	:	580 mg/m^3 per 15 minutes
Conversion factor	:	1 ppm = 7.097 mg/m^3 (101 kPa, 25ºC) 1 mg/m^3 = 0.141 ppm

De European Commission has not classified nickel carbonyl for effects on fertility. For effects on development, nickel carbonyl has been classified in category 2.

4.2 Human studies

Fertility

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

Developmental studies

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

Lactation

See chapter 7.

4.3 Animal studies

Fertility

No animal studies were found regarding the effects of exposure to nickel carbonyl on fertility.

Developmental studies

Inhalation

Pregnant Fischer rats were given a single exposure by inhalation of 0.06 (group A) or 0.12 mg $Ni(CO)_4$/l (60 or 120 mg/m^3) for 15 min (group B) on day 8 of gestation (Sun78a, abstract). The dams were killed on the 20th day of gestation. The number of live foetuses per dam and the body weights of these foetuses were significantly lower in group B than in the control group (no effects were found in group A). The ratio of dead foetuses to conceptuses was significantly higher in group B compared to the control group (no effects in group A). The incidence of ophthalmic malformations, including unilateral and bilateral microphthalmia and unilateral anophthalmia, as statistically significantly increased in both treatment groups compared to the control group.

Sunderman *et al*. (Sun79) exposed Fischer 344 rats by a single inhalatory exposure to either (I) 0.16 (160 mg/m^3) and 0.30 mg $Ni(CO_4)$/l (300 mg/m^3) for 15 min on gestation day 7, or (II) 0.08 and 0.16 mg $Ni(CO_4)$/l (80 and 160 mg/m^3) for 15 min on gestation day 8 or (III) 0.16 mg $Ni(CO_4)$/l (160 mg/m^3) for 15 min on gestation day 9. In the 160 mg/m^3 (gestation day 7) group no maternal mortality was observed. In the 300 mg/m^3 (gestation day 7) group only 10 out of 19 dams were alive on day 20 (day of sacrifice). In both groups an increased number of foetuses with anophthalmia and microphthalmia was observed. No mortality was observed in dams exposed to 80 mg/m^3 on gestation day 8. Two out of 15 dams died in the 160 mg/m^3 group exposed on gestation day 8. In addition an increased number of fetuses with microphthalmia was

seen. The animals exposed on gestation day 9 (group 111) showed no maternal mortality and no foetuses with malformations were observed. Maternal toxicity was not presented.

In the same paper the effects of exposure of Fischer 344 rats to 0 and 0.30 mg $Ni(CO_4)$/l (300 mg/m^3) for 15 min on day 7 of gestation was described. The dams were allowed to litter and rear their pups. In the Ni-treated group a decreased number of live pups per litter and an increased number of live pups with anomalies (anophthalmia, microphthalmia) were observed. Furthermore, pup weights were decreased 4 and 16 weeks postnatally. Maternal toxicity was not presented.

Sunderman *et al*. (Sun80) exposed Syrian hamsters by inhalation to 0.06 mg $Ni(CO_4)$/l (60 mg/m^3) for 15 min on gestation days 4, 5, 6, 7 or 8. Twenty-five percent of the dams exposed on day 4 and 49-59% of the dams exposed on day 5-8 died before day 15. An increased number of foetal abnormalities (exencephaly, haemorrhages into serous cavity) was observed after exposure on day 4 and 5. Only a few foetuses with abnormalities were noted after exposure on gestation day 6, 7 or 8.

In another experiment, described in the same paper (Sun80), Syrian hamsters were exposed by inhalation to a single dose of 0.06 mg $Ni(CO_4)$/l (60 mg/m^3) for 15 min on gestation day 5. Females were allowed to litter and rear their pups until postnatal day 65. Five of the exposed dams died before day 16. An increased neonatal mortality was observed (postnatal day 1-4). No behavioural or developmental abnormalities were detected in the progeny and no congenital malformations or pathological lesions were identified at autopsy (10 weeks postpartum).

Lactation

See chapter 7.

4.4 Conclusion

No papers were available on the effects of nickel carbonyl on fertility. Therefore, the committee recommends not to classify nickel carbonyl with respect to effects on fertility because of a lack of appropriate data.

No studies were available concerning the developmental effects of nickel carbonyl in man. Several papers of Sunderman *et al*. (Sun78a, Sun79, Sun80) reported ophthalmic malformations and lower birth weight in offspring after treatment of the dam with nickel carbonyl. However, in several dose groups maternal mortality was observed. Foetuses with anophthalmia and microphthalmia were observed (Sun79) after exposure of the dams to concentrations at which none of the dams died. However, additional maternal toxicity data were not presented and the animals were exposed only once.

Therefore, the committee proposes to classify nickel carbonyl with respect to effects on development in category 3 (substances which cause concern for humans owing to possible developmental toxic effects) and to label with R63 (Possible risk of harm to the unborn child).

Proposed classification for fertility

A lack of appropriate data precludes assessment of nickel carbonyl for fertility.

Proposed classification for developmental toxicity

Category 3, R 61.

Proposed labeling for effects during lactation

See chapter 7.

Chapter 5

Soluble nickel salts

5.1 Properties

Name	:	nickel nitrate
CAS-no	:	13138-45-9
Use	:	intermediate in the manufacture of nickel catalysts and nickel-cadmium batteries
Mol weight	:	182.70
Chem formula	:	$Ni(NO_3)_2$

Name	:	nickel sulphate
CAS-no	:	7786-81-4
Use	:	solid for electroplating
Mol weight	:	154.75
Chem formula	:	$NiSO_4$

Name	:	nickel chloride
CAS-no	:	7718-54-9
Use	:	solid for electroplating
Mol weight	:	129.60
Chem formula	:	$NiCl_2$

Name	:	nickel acetate
CAS-no	:	6018-89-9
Use	:	solid for electroplating
Mol weight	:	189.80
Chem formula	:	$NiCH_2COOH.4H_2O$

5.2 Human studies

Fertility

No studies were found regarding the effects of exposure to soluble nickel salts on human fertility.

Developmental studies

Chashschin *et al*. (Cha94) compared the health and reproductive and congenital effects in females (nickel exposed and non-exposed (construction) workers). The females were employed in a nickel hydrometallurgy refining plant and exposed to a mixture of nickel compounds. The concentration of $NiSO_4$ aerosols measured was 0.08-0.31 mg/m^3. In the nickel-exposed group (n=290), threatening and spontaneous abortion occurred at 17.2 and 15.9% whereas in the control group (n=336) these percentages were 7.6 and 8.5%. Complications (not specified) were also recorded more often in the pregnancies of nickel-exposed workers, 29.7 versus 20.6%. Incidences of structural malformations were higher among infants of Ni-exposed female workers than in infants of controls (16.9 versus 5.8%). The relative risks for all kinds of effects, defects of the cardiovascular system and for musculoskeletal defects were 2.9, 6.1 and 1.9, respectively. However, as the exact exposure remained unclear and limited data were available on statistical and sampling details of the pregnancies, new-born babies and the control group, the committee considered the quality of this study to be insufficient for classification.

Lactation

See chapter 7.

5.3 Animal studies

Fertility

Inhalation

In 1996, the NTP published three toxicology and carcinogenesis studies on nickel subsulfide, nickel oxide and the soluble nickel sulfate hexahydrate (NTP96a,b,c). F344/N rats and B6C3F1 mice were exposed to nickel sulfate hexahydrate by inhalation for 16 days, 13 weeks or 2 years. Rats and mice (male and female) were exposed to 0, 3.5, 7, 15, 30 and 60 mg nickel sulphate hexahydrate/m3 during 16 days (12 days of exposure). At the end of the experiment mortality was observed in the highest dose groups in both males and females. No effects were found on fertility. In addition, no effects on fertility, sperm numbers, reproductive tissues weight and estrous cycle length were observed in the 13 week study in rats and mice. Predominantly, respiratory tract toxicity was observed at low concentrations in rats and mice. Finally, no effects were observed on fertility in rats and mice after two years of exposure.

Gavage

Waltschewa *et al*. (Wal72) dosed male white rats by gavage for 120 days with 25 mg $NiSO_4$/kg body weight and found effects on spermiogenesis. At the end of the test period, treated animals were caged together with untreated female rats in oestrus. In the female animals, mated with exposed males, no fertilization occurred. Slight necrosis in liver and kidney were characterised by the authors as small and non-specific.

Pandey *et al*. (Pan99) found effects on the relative weights of testes, seminal vesicles and prostate gland after exposure by gavage of mice to 5 or 10 mg $NiSO_4$/kg bw/day 5 days per week for 35 days. Sperm motility and total sperm count were significantly decreased in the high dose group, whereas the number of morphological abnormalities was increased in both exposed groups. In the high dose groups, several enzyme activities were altered and histopathological changes were found.

In the same paper (Pan99), a study was described in which male mice treated during 35 days with 10 mg $NiSO_4$/kg bw/day by gavage were mated with untreated females; caesarian section was performed on day 18 of gestation. The fertility index was 46.6% after treatment compared to 66.6% in the controls. The number of implantations and live foetuses was decreased, whereas pre-implantation loss, number of resorptions and post-implantation loss were increased after treatment. No effects were observed on the

number of corpora lutea. In both studies, mortality or effects on body weight were not observed, however animals of the high dose group showed alopecia and sluggishness.

Dermal

Mathur *et al*. (Mat77b) studied the effects of $NiSO_4$ in male albino rats, after daily dermal exposure to 0, 40, 60 and 100 mg Ni/kg body weight for 30 days. Animals were killed after 15 and 30 days of Ni exposure. No effects on the testis were observed after 15 days. After 30 days tubular damage and lumen filled with degenerated sperms and oedematous fluid were observed in the testis of the 60 mg/kg group. In the testis of the 100 mg/kg group increased tubular degeneration and oedema and distorted epithelium of the seminiferous tubules were observed. Skin of $NiSO_4$ painted rats showed hyperkeratinisation, vacuolisation, hydropic degeneration of basal layer and atrophy of epidermis after 30 days with increasing severity at increasing dose. The liver showed areas of focal necrosis, congestion and dilatation of sinusoids after exposure for 30 days at the two highest concentrations. No clinical symptoms of poisoning and no mortalities were observed.

Drinking water

Smith *et al*. (Smi93) studied in female Long-Evans rats the potential effects of exposure to $NiCl_2$ (0, 10, 50 and 250 mg Ni/l) in drinking water during premating (11 weeks), mating and two successive gestation and lactation periods. Males were unexposed. No effects on female fertility were observed in the treated groups.

Male and female Wistar rats were exposed to $NiCl_2.6H_2O$ in drinking water (Kak99). In the first experiment female rats were exposed to 10, 30 or 100 mg Ni/l for 14 days or to 30 mg Ni/l for 100 days prior to mating with untreated males. In the second experiment, male rats were exposed to 30 mg Ni/l for 28 or 42 days prior to mating with untreated females. Finally, in the third experiment both males and females were treated with 30 mg Ni/l 28 days prior to mating. Pregnant females were exposed during the gestation and lactation period at the same level as before mating. The fertility index (number of pregnancies/copulated females) decreased with 50%, in exposed (28 days prior to mating) and non-exposed female rats both mated with exposed male rats (exposed 28 day prior to mating). When male rats were exposed 42 days before mating to untreated females, the fertility index decreased with 17%. The gestation index (pups born alive/female) was significantly lower when males were exposed for 28 days before mating intreated female rats. Length of gestation did not differ significantly between groups. In the males, the mean diameters of the seminiferous tubules were significantly smaller than those of the control group both after 28 and 42 days of exposure prior to

mating. In addition, the males exposed for 28 days had fewer basal spermatogonia per 250 µm along the outer edge of the tubules than the control males and the ratio closed/ open tubules had significantly increased.

Diet

Ambrose *et al*. (Amb76) studied potential effects of $NiSO_4$ in the diet (0, 250, 500 and 1000 mg/kg) during a 3-generation study (2 litters per generation) in Wistar rats. No adverse effects on fertility were observed.

Intratesticular

Kamboj *et al*. (Kam64) demonstrated effects 7 days after a single intratesticular injection in albino rats of 0.08 mmol $Ni(NO_3)_2$/kg body weight (14.6 mg/kg body weight); testicular weight was decreased and focal necrosis was observed. Daily subcutaneous injections of $Ni(NO_3)_2$ (0.08 mmol/kg body weight/14.6 mg/kg body weight) in Swiss mice caused shrinkage of the tubules of the testis and spermatogenic arrest after 7 days. No data were presented on general toxicity in rats and mice.

Subcutaneous

Hoey (Hoe66) studied the effects of $NiSO_4$ on the testis of male albino rats after a single subcutaneous dose or after daily injections during 30 days of 0.04 mmol/kg body weight (6.2 mg/kg body weight). $NiSO_4$ produced acute and chronic changes in the histology of the testis and interfered, to some degree, with spermatogenesis. No data were presented on other toxicity parameters in the animals.

Intraperitoneal

Mathur *et al*. (Mat77a) studied the effects of $NiSO_4$ after daily intraperitoneal injections for 90 days of 3 mg Ni/kg body weight in male albino rats. Animals were killed at 2, 7, 15, 30, 60 and 90 days intervals. After 30, 60 and 90 days an increased level of adenosine triphosphatase was found in the testis and a decreased level of acid phosphatase was observed after 60 and 90 days. Only after 90 days degenerative changes were seen in a few seminiferous tubules. Five out of 30 treated animals died of an unknown cause during the course of the study. No gross pathological abnormalities were observed in testis and myocardium, but mild congestion was found in liver and kidney after 60 and 90 days. After 90 days necrosis was observed in liver, kidney and myocardium.

Deknudt and Léonard (Dek82) studied the effects of a single intraperitoneal injection of $NiCl_2$ and $Ni(NO_3)_2$ (12 mg Ni/kg body weight) in a dominant lethal test in BALB/c mice. Six hours after treatment males were mated with untreated females at weekly intervals for 5 weeks. Treatment with $NiCl_2$ and $Ni(NO_3)_2$ induced a decreased incidence of pregnant females from week 1-4 for $NiCl_2$ and from week 1-5 for $Ni(NO_3)_2$. A decrease in the mean number of implantations per female was observed for both test substances from the second up to the fourth week of mating. No effect on post-implantation loss was observed. One and two out of 40 animals treated with $NiCl_2$ and $Ni(NO_3)_2$, respectively, died after one week of treatment.

Xie *et al*. (Xie95) injected male ICR mice intraperitoneally with $NiCl_2$ (0.5, 1.0, 3.0 and 5 mg Ni/kg body weight). Twenty-four hours after administration, lipid peroxidation and concentrations of Ni, Ca and Fe in the testis were all increased in a dose-dependent manner. After a single intraperitoneal injection of $NiCl_2$ (5 mg Ni/kg body weight) lipid peroxidation in the testis significantly increased at 6 h compared to the control and started to decrease after attaining peak values at 2 days post-injection. The testicular weight decreased gradually with time, and significantly decreased 3 days after Ni administration. Treatment with $NiCl_2$ (5 mg Ni/kg body weight, intraperitoneally) reduced the fertility rate (number of virgin females impregnated versus number of females tested) of male mice to 50%, while treatment with 1 mg Ni/kg body weight reduced the fertility rate to 80%. Treatment with 0.5 mg Ni/kg body weight did not affect the fertility of male mice.

Developmental

Gavage

Male mice were treated during 7 weeks with 10 mg $NiSO_4$/kg bw/day by gavage and mated with untreated females; caesarian section was performed on day 18 of gestation. Foetal weight was decreased, whereas no effects were observed on the foetal crown-rump length. Mortality or effects on body weight were not observed, however, treated animals showed alopecia and sluggishness (Pan99).

Drinking water

Smith *et al*. (Smi93) studied the effects in female Long-Evans rats after exposure to $NiCl_2$ (0, 10, 50 and 250 mg Ni/l) in drinking water during premating (11 weeks), mating and two successive gestation and lactation periods. Males were unexposed. In the 10 mg/l group, an increased number of dead pups was observed on postnatal (PN) day 1 in the second lactation period. In the 50 mg/l group, a reduced weight gain of male

pups during the first lactation period was observed. In dams of the high dose group several effects were observed: a decreased liquid consumption during prebreeding and both gestation periods, increased food consumption/kg body weight during prebreeding, reduced weight gain during the first gestation period, decreased plasma prolactin levels and an increased number of dead pups on PN 1 and 21 of both lactation periods. Pup body weights and plasma prolactin levels were unchanged. Throughout the study there were no overt clinical signs of toxicity in any of the groups.

Male and female Wistar rats were exposed to $NiCl_2.6H_2O$ in drinking water (Kak99). In the first experiment female rats were exposed to 10, 30 or 100 mg Ni/l for 14 days or to 30 mg Ni/l for 100 days prior to mating with untreated males. In the second experiment, male rats were exposed to 30 mg Ni/l for 28 or 42 days prior to mating with untreated females. Finally, in the third experiment both males and females were treated with 30 mg Ni/l 28 days prior to mating. Pregnant females were exposed during the gestation and lactation period at the same level as before mating. One of the females of the 100 mg/l exposure group ate all three of her pups and died soon after this (cause unclear). The litter size at 21 days (weaning) was significantly decreased in the groups in which a) the females were exposed to 100 mg/l and mated with untreated males, b) the males were exposed to 30 mg/l during 28 days prior to mating with untreated females and c) both the males and females were exposed. No significant differences in body weight of the pups at weaning were observed between the groups. Pups that died during lactation were runts: their heads were disproportionately large, the posteriors of the bodies were underdeveloped and they moved slowly. The pups of the dams exposed to 100 mg/l had short and sparse hair, especially on the sides and posterior part of the back. The pups of the dam that died during nursing were oedemic. The relative weight of the liver of females exposed to 100 mg/l had decreased. No other differences in body weight, relative weight of liver or kidneys in pups or dams were observed. Ni accumulated most in kidney, followed by liver and skin, both in dams and in pups. Ni exposure did not change the Zn and Cu levels in the tissues of pups very much.

Diet

Ambrose *et al*. (Amb76) studied 3 generations of Wistar rats that were fed diets containing Ni (as $NiSO_4$) in concentrations of 250, 500 and 1000 mg/kg. An increased incidence of stillborn pups was found in the first generation at all dietary dose levels of Ni. The mean body weight of weaned pups had decreased in each of the 3 generations of rats of the 1000 mg/kg group. Gross observations on pups showed no teratogenic effects. Animals of the F_0 generation fed with 1000 mg/kg exhibited slightly lower body weights (females: 8%, males: 13%).

Intravenous

Ferm (Fer72) administered $NiCH_2COOH$ at dosages, ranging from 0.7-10 mg Ni/kg body weight, to pregnant golden hamsters by intravenous injection on day 8 of gestation. Dose-related increases in the number of resorptions, as well as a few unspecified malformations in surviving embryos were observed. No data on maternal toxicity were available.

Intramuscular

Sunderman *et al*. (Sun78b) studied the embryo- and foetotoxicity in Fischer 344 rats of $NiCl_2$ when injected intramuscularly (8, 12 or 16 mg Ni/kg body weight) on day 8 of gestation. On day 20 of gestation a reduced number of live foetuses was found in the mid and high dose group and reduced foetal body weight in the high dose group. No skeletal or visceral anomalies were observed. In a study in which the rats were injected intramuscularly with 16 mg Ni/kg body weight on gestation day 8 and the females were allowed to deliver and raise their pups, a reduced number of live pups was born and pup weight was reduced from week 4-8. No specific behavioural or developmental abnormalities were observed. No maternal toxicity occurred. When $NiCl_2$ was administered in repeated intramuscular doses of 1.5 or 2.0 mg Ni/kg body weight, twice daily, on days 6-10 of gestation, the higher dose caused significant intrauterine mortality, but did not cause any reduction in the mean body weight of live pups. No skeletal or visceral anomalies were observed. No dams died during the course of the study.

In the same paper (Sun78b) the results were reported of a study with rats treated intramuscularly on day 18 of gestation with a single dose of $NiCl_2$ at doses of 6, 8 and 16 mg Ni/kg body weight. In the high dose group the number of dead foetuses was markedly increased. At this dose level half of the number of dams died within 24 hours.

Intraperitoneal

Lu *et al*. (Lu79) observed a dose-related increase in foetal deaths, a decreased foetal weight, delay in skeletal ossification and a higher incidence of abnormalities (acephaly, exencephaly, cerebral hernia, open eyelids, cleft palate, ankylosis of extremity, club foot and micromelia) in pregnant CD-1 mice following a single intraperitoneal injection of $NiCl_2$ (1.2, 2.3, 3.5, 4.6, 5.7 or 6.9 mg Ni/kg bodyweight) between days 7 and 11 of gestation. After treatment at the highest dose on day 7 or 8 one dam died, whereas from the animals treated on day 9, 10 and 11 several animals died which were treated with 4.6 mg Ni/kg body weight or more.

Pregnant female albino mice (NMRI/Bom) were treated with a single intraperitoneal injection of 20 mg $NiCl_2.6H_20$/kg body weight on day 0, 1, 2, 3, 4 or 5 of gestation (Sto81). Only after treatment on day 0 the average number of implantations per dam was decreased. All treatments resulted in higher resorption frequencies. The size of the litters in the control groups was larger than that of the Ni treated dams on day 0, 2 and 4 of gestation. Lower average body weights of the live foetuses were observed after treatment on day 0, 1, 2, 3 and 4. Significant differences in the number of abnormalities and dead foetuses between control and Ni treated groups were found. Abnormalities included haematomas, exencephaly, anaemic appearance and retarded development.

Mas *et al*. (Mas85) studied the effects of a single intraperitoneal injection of $NiCl_2$ (0, 1, 2 and 4 mg Ni/kg body weight) on gestation days 8, 12, or 16 in Wistar Porton rats. On gestation day 8, a dose-related increase in the number of foetuses with haemorrhages over the four dosage levels was observed. Hydronephrosis was caused by treatment with 2 and 4 mg/kg and an increased number of foetuses with hydrocephalus was observed after treatment with 4 mg/kg. In all groups exposed to $NiCl_2$ on day 12 of gestation an increased number of malformed foetuses (hydrocephalus, haemorrhages, hydronephrosis) was observed. In the two highest groups foetal weight was reduced significantly. After exposure on day 16 no effects were noted. The number of pregnant females per group at sacrifice on day 20 of gestation was very low in this study (range 1-6). The control group (8, 12 and 16 days) consisted of 12 dams.

Diwan *et al*. (Diw92) treated pregnant F344/NCr rats with $NiCH_2COOH.4H_2O$ intraperitoneally either once on day 16 of gestation (90 µmol/kg body weight (17.1 mg/kg body weight, group 1)) or twice on day 15 and 17 of gestation (45 µmol/kg body weight/day (8.5 mg/kg body weight, group 2)). Control rats received $NaCH_2COOH$ (180 µmol/kg body weight) on day 17 of gestation. Offspring of these rats received either ordinary tap water (1A and 2A) or drinking water containing the promotor Na barbital (500 mg/l) (2A and 2B) during weeks 4-85 days of age. The body weights of male offspring in all treatment groups were significantly lower at 75 weeks of age compared to the control group. Renal cortical epithelial and renal pelvic transitional epithelial tumours occurred only in male offspring of the $NiCH_2COOH$ and Na barbital treated group significantly more compared to the control group. Pituitary tumour incidence was significantly higher in offspring of both sexes given Ni prenatally. Pituitary tumours appeared much earlier and often were malignant. All offspring of a group of pregnant rats which received four intraperitoneal injections of $NiCH_2COOH$ (45 µmol/kg body weight (8.5 mg/kg body weight)) on day 11, 13, 15 and 17 of gestation died within 72 h after birth.

Lactation

See chapter 7.

5.4 Conclusion

No human studies were available concerning the potential effects on fertility. Six animal studies were available regarding possible effects on fertility after exposure to soluble nickel salts via exposure routes which the committee considers relevant to man. Waltschewa *et al*. (Wal72) and Mathur *et al*. (Mat77b) found effects on spermiogenesis after exposure to $NiSO_4$ by gavage and via the skin, respectively, but in the presence of parental toxicity. Ambrose *et al*. (Amb76) did not find effects on fertility after exposure to $NiSO_4$ via the diet.

The NTP did not find effects on fertility in female and male rats and mice after exposure to nickel sulfate hexahydrate by inhalation. Smith *et al*. (Smi93) found no effects on female fertility in rats after exposure to $NiCl_2$ in drinking water up to two generations. In contrast, Käkela *et al*. (Kak99) observed a decrease of 50% in the fertility index when males were exposed to $NiCl_2.6H_2O$ in drinking water and mated with untreated females or when both males and females were exposed for 28 days prior to mating. However, these effects were observed in the presence of decreased female body weight; after exposure of the males for a longer period (42 days), the effects were less strong. This was also true for the effects found on the gestation index and on spermatogenesis.

Pandey *et al*. (Pan99) found effects on the relative weights of testis, seminal vesicles and prostate gland after exposure of mice to $NiSO_4$ by gavage in the absence of general toxic effects. In addition, the number of morphologically abnormal spermatozoa was increased. In the presence of paternal toxicity (alopecia and sluggishness), the fertility index, number of implantations and live foetuses were decreased and pre-implantation loss, number of resorptions and post-implantation loss were increased. Similar findings were observed in several other studies with a less relevant exposure route.

Therefore, on the basis of the male-mediated effects in Pan99, the committee proposes to classify soluble nickel salts in category 3 (substances which cause concern for human fertility) and to label these compounds with R62 (possible risk of impaired fertility).

No human studies were available concerning the potential effects on development. Four animal studies were available regarding developmental possible effects of soluble nickel salts with exposure routes relevant to man. Pandey *et al*. (Pan99) found a decrease in foetal mice weight after exposure of sires to $NiSO_4$ by gavage. However, the exposed

males suffered from alopecia and sluggishness. After exposure of rats (dams and sires) to $NiCl_2$ via drinking water, an increased number of dead pups, a decreased litter size at weaning and reduced weight gain of the male pups were observed in the absence of parental toxicity (Smi93, Kak99). Pups that died during lactation were runts (Kak99). In addition, an increased incidence of stillborn pups and a decreased mean weight of weaned pups were observed after exposure to $NiSO_4$ via the diet in the absence of maternal toxicity (Amb76).

In conclusion, in animal studies clear developmental toxicity was observed in the absence of parental toxicity using relevant exposure routes. Therefore, the committee recommends to classify soluble nickel salts in category 2 (substances which should be regarded as if they cause developmental toxicity in humans) and to label the compounds with R61 (may cause harm to the unborn child).

Proposed classification for fertility

Category 3, R62.

Proposed classification for developmental toxicity

Category 2, R 61.

Proposed labelling for effects during lactation

See chapter 7.

Chapter 6

Insoluble nickel salts

6.1 Properties

Name	:	nickel oxide
CAS-no	:	1313-99-1; 11099-02-08; 34492-97-2
Use	:	stainless steel welding, mining, used to make nickel catalysts and in the ceramics industry, air pollutant
Mol weight	:	74.69
Chem formula	:	NiO

Name	:	nickel subsulfide
CAS-no	:	12035-72-2
Use	:	refinary processes
Mol weight	:	240.19
Chem formula	:	Ni_3S_2

6.2 Human studies

Fertility

Jelnes *et al*. (Jel88) compared the semen quality of steel welders and reference persons, mainly non-welders from the same plant. No differences were found in semen volume, sperm concentration, percentages live, immotile and normal sperm. In addition, no differences in the same parameters were observed between stainless steel welders using manual metal arc welding (MMA) and non-welders employed at plants without welding.

Development

No studies were found regarding the effects of exposure to insoluble nickel compounds on human development.

Lactation

See chapter 7.

6.3 Animal studies

Fertility

F344/N rats and $B6C3F_1$ mice were exposed by inhalation to nickel subsulfide (0, 0.6, 1.2, 2.5, 5.0, 10.0 mg/m^3) for 6 hours per day for 12 days (not including weekends) (Ben87). Two male rats and all mice died after exposure to 10 mg/m^3. Degeneration of the testicular epithelium was observed in both rats and mice exposed to 5 and 10 mg Ni_3S_2/m^3 (who survived the exposure). Degeneration of the respiratory epithelium and atrophy of the olfactory epithelium of the nose occurred in rats exposed to at concentration exposed to nickel subsulfide at concentration as low as 0.6 mg.

In 1996, the NTP published three toxicology and carcinogenesis studies of nickel subsulfide, nickel oxide and the soluble nickel sulfate hexahydrate (NTP96a,b,c). F344/N rats and $B6C3F_1$ mice were exposed to nickel subsulfide or nickeloxide by inhalation for 16 days, 13 weeks or 2 years. Rats and mice (male and female) were exposed to 0, 1.2, 2.5, 5, 10 and 30 mg nickel oxide/m^3 or 0, 0.6, 1.2, 2.5, 5 and 10 mg nickel subsulfide/m^3 during 16 days (12 days of exposure). No effects were found on sperm numbers or histology of the testes in the 16-day experiment. In addition, no effects on fertility, sperm numbers, reproductive tissues weight and estrous cycle length were

observed in the 13-week study in rats and mice, except for the concentration of epididymal spermatozoal which was significantly decreased after 10 mg nickeloxide/ m^3. However, the committee is of the opinion that this effect is due to the (unusual) high epididymal spermatozoal concentration in the control group. Predominantly, respiratory tract toxicity was observed at low concentrations in rats and mice. Finally, no effects were observed on fertility parameters in rats and mice after two years of exposure.

Development

Female Wistar rats were exposed to 0.8, 1.6 and 3.2 mg Ni/mmg/m^3 (NiO) during the whole gestation period (Wei80). In all dose groups the body weight gain of the dams was reduced and the weight of the lungs had increased. In addition, in the two highest dose groups the weight of the kidneys and the number of erythrocytes were decreased and haematocrit and mean cell volume of erythrocytes (MCV) were increased. The number of leukocytes was only increased in the lowest dose group and the concentration of urea in serum in the highest dose group was decreased. Haemoglobin, alkalinephosphatase in serum and urine, bilirubin, urea in urine and weight of livers were not significantly changed in dams. The body weight of the foetuses was reduced in the groups receiving 1.6 and 3.2 mg/m^3. Leukocytes and urea in serum of the group receiving 1.6 mg/m^3 were significantly enhanced. Numbers of foetuses and placentas, weight of placentas, haemoglobin, haematocrit, erythrocytes, MCV (mean cell volume), alkaline phosphatase in serum did not significantly differ from controls.

The effects of intramuscular injection of Ni_3S_2 (80 mg Ni/kg body weight) in Fischer F344 rats on gestation day 6 were studied by Sunderman *et al* (Sun78b). The exposure to Ni_3S_2 reduced the mean number of live foetuses per dam. No skeletal or visceral anomalies were observed. None of the dams died within 2 weeks after the intramuscular injection of Ni_3S_2. Maternal toxicity was not presented.

Pregnant female albino Fischer 344 rats were given a single intramuscular injection of 20 mg Ni_3S_2 on day 6 of gestation (Sun81). The dams delivered their litters on days 21 or 22 of gestation and no difference was observed in the number of pups between the control and the treated groups, however the body weights of the pups of the treated dams were lower. No significant effects upon the incidence of tumours in the progeny of either treated or control dams were observed, nor any differences in incidence of non-neoplastic lesions or cumulative mortality rate.

Lactation

See chapter 7.

6.4 Conclusion

Jelnes *et al*. (Jel88) found no effect on semen quality of stainless steel welders. No other relevant data were available to assess the toxic effects of exposure to insoluble nickel compounds with respect to fertility in man. Testicular degeneration was observed in rats and mice, at exposure levels which caused severe general toxicity as well (Ben87). No effects on fertility were found by the NTP (NTP96a,b,c). Therefore, the committee recommends not to classify insoluble nickel compounds with respect to effects on fertility because of a lack of appropriate data.

Sunderman *et al*. (Sun78b, Sun81) studied the developmental effects of Ni_3S_2 in rats after intramuscular injection, which is a less relevant route of exposure for humans. Moreover, maternal toxicity was not recorded and contradictory results were obtained. Weischer *et al*. (Wei80) found a decrease in the body weight of foetuses after inhalatory exposure of the pregnant dams to nickel oxide. However, in all dose groups maternal toxicity was present. Therefore, the committee recommends not to classify insoluble nickel compounds with respect to effects on development because of a lack of appropriate data.

Proposed classification for fertility

A lack of appropriate data precludes assessment of insoluble nickel compounds for fertility.

Proposed classification for developmental toxicity

A lack of appropriate data precludes assessment of insoluble nickel compounds for development.

Proposed labelling for effects during lactation

See chapter 7.

Chapter 7

Lactation

7.1 Human studies

Human milk samples (n=179) from three different countries and three different social groups were freeze dried and analysed for Ni content (Cam82). The age of the infants at the time of breast milk sampling was between 3 and 9 months. The amount of Ni ranged between 0.03-0.42 µg/g freeze dried material (comparable to 3-42 µg/l breast milk). No significant differences in concentrations occurred between countries or groups.

Ni was measured in 46 samples of human milk collected from 13 women between delivery and 38 days postpartum (Cas87). Ni concentrations did not change with time; the overall mean was 1.16 ± 0.41 µg/l (range 0.52-2.04 µg/l).

Biego *et al*. (Bie98) determined the concentrations of Ni in breast-milk, bottled, dried and evaporated milk to be below 2.9 µg/l.

Friel *et al*. (Fri99) examined the Ni concentration in human milk up to 3 months post partum. Median values ranged from 0 to 28 µg/l and no differences were found between the Ni concentration in milk from mothers of premature (n=24) or of full term infants (n=19).

The maximum safe level for Ni (all compounds), based on an ADI of 5 µg/kg bw/day (Jan94) is 25 µg Ni/l breast milk (see Annex E for calculations).

7.2 Animal studies

By injecting pregnant mice intraperitoneally with 0.14 mg ^{63}Ni/kg body weight as $NiCl_2$ on day 18 of gestation and on day 0 and 3 of lactation, Jacobsen *et al*. (Jac78) showed that Ni is transported over the placenta and is excreted in milk.

Dostal *et al*. (Dos89) treated lactating Sprague-Dawley (CD) rats with daily subcutaneous injections of 50 or 100 µmol $NiCl_2$/kg body weight (6.5 or 13.0 mg/kg body weight) on days 12 to 15 of lactation. No significant effects on maternal body weight, liver weight or hepatic lipid peroxidation were observed. Thymus weight was significantly reduced at 100 µmol/kg (13.0 mg/kg) and food consumption was significantly decreased by 18 and 33% at doses of 50 and 100 µmol $NiCl_2$/kg (6.5 and 13.0 mg/kg), respectively. The composition of the milk was significantly altered by the $NiCl_2$ treatment (increase in milk solids and lipid, decrease in milk lactose), milk synthetic activity was reduced by $NiCl_2$. Average milk Ni concentrations were 513 and 1030 µg/l 4 h after the last dose of 50 or 100 µmol/kg body weight, respectively. Pups of lactating rats treated with 50 or 100 µmol $NiCl_2$/kg had Ni plasma concentrations of 24 and 50 µg/l, respectively. Liver weights of both male and female pups were decreased at the highest dosing rate, either due to Ni exposure or changes in milk composition.

7.3 Conclusion

Some concentrations of Ni found in human breast milk as described above exceed the calculated safe level of 25 µg Ni/l breast milk (see Annex E). However, it is not possible to distinguish which nickel compound(s) is(are) causing these high concentrations as the source of exposure is not described.

In rats, it was shown that $NiCl_2$ (a soluble nickel salt) impairs milk quality and quantity, resulting in reduced liver weights of the pups (Dos89). On the basis of the comparable physico-chemical characteristics of soluble nickel salts, the committee recommends to label these compounds with R64 (May cause harm to breastfed babies). The committee recommends not to label metallic nickel, nickel carbonyl and insoluble nickel compounds for effects during lactation due to a lack of appropriate data.

Proposed labelling for effects during lactation for metallic nickel

A lack of appropriate data precludes assessment of metallic nickel for labelling for effects during lactation.

Proposed labelling for effects during lactation for nickel carbonyl

A lack of appropriate data precludes assessment of nickel carbonyl for labelling for effects during lactation.

Proposed labelling for effects during lactation for soluble nickel salts

R64.

Proposed labelling for effects during lactation for insoluble nickel compounds

A lack of appropriate data precludes assessment of insoluble nickel compounds for labelling for effects during lactation.

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A	De adviesaanvraag
B	De commissie
C	Directive (93/21/EEC) of the European Community
D	Fertility and developmental toxicity studies
E	Calculation safe levels of nickel in (human) breast milk

Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
  Toxicologist, Institute for Risk Assessment Sciences, Utrecht
- AM Bongers, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- HFP Joosten
  Toxicologist, NV Organon, Department of Toxicology and Drug Disposition, Oss
- D Lindhout
  professor of Medical Genetics, paediatrician; UMC, Utrecht
- JHJ Copius Peereboom-Stegeman
  Toxicologist, Catholic University Nijmegen, Nijmegen
- N Roeleveld
  Epidemiologist; Catholic University Nijmegen, Nijmegen
- AH Piersma
  Reproductive toxicologist, National Institute of Public Health and the Environment, Bilthoven
- DH Waalkens-Berendsen
  Reproductive toxicologist, TNO Nutrition and Food Research, Zeist
- PJJM Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present document was prepared by MEM Kuilman and DH Waalkens-Berendsen, from the TNO Nutrition and Food Research Institute in Zeist, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: A Aksel.
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 2002. The following persons and organisations have commented on the draft document:

- RD Zumwalde
  National Institute for Occupational Safety and Health, USA
- A Aalto
  Ministry of Social Affairs and Health, Finland

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 with soluble nickel salts: Inhalation.

authors	species	experimental period/design	dose	general toxicity	effects on reproductive organs/ effects on reproduction	rem.
NTP 1996 (a)	Male and female F344/N rats (N=5/group)	16 days:12 days exposure, 6 h/d, 5 d/w	0, 3.5, 7, 15, 30, 60 mg $NiSO_4.6H_2O$/ m^3	60 mg/m^3: 2 males died and all females died.30 mg/m^3: one female died. All body weight decreased (except in controls). respiratory tract toxicity in all dose groups	No effects on sperm number and histology of testes	
		13 weeks 6 h/d, 5 d/w	0, 0.12, 025, 0.5, 1, 2 mg $NiSO_4.6H_2O$/ m^3	2 mg/m^3: one male died. No effects on body weight respiratory toxicity	No effects on sperm number and histology of testes, estrous cycle length	
		2 years, 6 h/d, 5 d/w	0, 0.12, 0.25, 0.5 mg $NiSO_4.6H_2O$/ m^3	No mortality; respiratory tract toxicity	no effects	
	Male and female B6C3F1 mice (N=5/group)	16 days:12 days exposure, 6 h/d, 5 d/w	0, 3.5, 7, 15, 30, 60 mg $NiSO_4.6H_2O$/ m^3	7 mg/m^3 and higher: all mice died; all body weights decreased (except in control) respiratory toxicity	No effects on sperm number and histology of testes	
		13 weeks 6 h/d, 5 d/w	0, 0.12, 025, 0.5, 1, 2 mg $NiSO_4.6H_2OO$/ m^3	4 control males and 3 females died;1.2 mg/m^3: 1 male died; No effects on bodyweight respiratory toxicity	No effects on sperm number and histology of testes, estrous cycle length	
		2 years, 6 h/d, 5 d/w	0, 0.25, 0.5, 1 mg $NiSO_4.6H_2O$/ m^3	No mortality; respiratory tract toxicity	no effects	

Table 1.2 Fertility studies with soluble nickel salts: Gavage.

authors	species	experimental period/design	dose	general toxicity	effects on reproductive organs/ effects on reproduction	remarks
Waltschewa *et al*. (1972)	male white rats (n=30)	daily treatment during 120 days premating, thereafter mating with untreated females	25 mg $NiSO_4$/kg bw	slight necrosis in liver and kidney (characterised as small and non-specific by the authors)	severe lesions in sperm cells particularly in spermiogenesis no fertilization in exposed group	males, 5 months of age, mean bw 120 g
Pandey *et al*. (1999)	male albino mice (Swiss) (n=20)	1) treatment 5 days a week during 7 weeks 2) treatment 5 days a week during 7 weeks mating with non-treated females (1:3), on 18th day of gestation laparotomies were performed	1) 5 and 10 mg $NiSO_4$/kg bw/day by 2) males 10 mg $NiSO_4$/kg bw/day , females were not treated	no mortality or effect on body weight gain, animals of the high dose showed alopecia and sluggishness	1) both the relative and the absolute weight of the testis were significantly decreased in both exposure groups, only the relative weight of the epididimis had significantly decreased in the high dose group, the weights of the seminal vesicles and prostate gland were significantly decreased, absolute (high dose) and relative (low and high dose)sperm motility and total sperm count were significantly decreased in the high dose group; the number of morphological abnormalities was increased in both exposed groups -glutamyl transpeptidase (+), sorbitol (-) and LDH (+) were significantly altered in the high dose groupsevere histopathological changes in the high dose group as well as accumulation of nickel in testis, epidimydis and seminal vesicle in both exposed groups were observed2) fertility index was 46.6% after treatment compared to 66.6% in the controls; no differences in the number of corpora lutea and foetal crown-rump length was found; the number of implantations, live foetuses and foetal weight were decreased, whereas pre-implantation loss, number of resorptions and post-implantation loss were increased after treatment	general: effects on foetal crown-rump length and foetal weight are considered developmental effects

Tabel 1.3 Fertility studies with soluble nickel salts.

authors	species	experimental period/design	dose	general toxicity	effects on reproductive organs/ effects on reproduction	rem.
Diet						
Ambrose *et al*. (1976)	male and female Wistar rats (n=25)	premating, mating, gestation and lactation during 3-generations	0, 250, 500 and 1000 mg Ni/kg feed ($NiSO_4$)	slightly lower body weights of F0 at 1000 mg/kg	no effects on fertility	
Dermal						
Mathur *et al*. (1977b)	male albino rats (n=8)	daily treatment for 30 days	0, 40, 60 and 200 mg Ni/kg bw ($NiSO_4$)	skin with dose dependent hyperkeratinisation, vacuolisation, hydropic degeneration of basal layer and atrophy of epidermis and liver with focal necrosis, congestion and dilatation of sinuoids at 60 and 100 mg/kg bw after 30 days. No clinical symptoms of poisoning or mortality	no effects on testis after 15 days of treatmentafter 30 days tubular damage and degenerated sperms and oedematous fluid in lumen at 60 mg/kg bw and increased effects as well as distorted epithelium of the seminiferous tubules at 100 mg/kg bw	
Drinking water						
Smith *et al*. (1993)	female Long-Evans rats (n=34)	females during premating (11w), mating, gestation and lactation, males were unexposed, sacrifice after L2	0, 10, 50 and 250 mg Ni/l ($NiCl_2$) water	no effects observed	no effects on fertility	

Table 1.4 Fertility studies with soluble nickel salts.

authors	species	experimental period/design	dose	general toxicity	effects on reproductive organs/ effects on reproduction	remarks
Drinking water						
Käkelä *et al*. (1999)	male and female Wistar rats (n= 6)	males, females or both premating, females gestation, lactation	F: 10, 30 or 100 mg Ni/l 14 days or 30 mg Ni/l 100 days prior to mating M: 30 mg Ni/l 28 or 42 days prior to mating F+M: 30 mg Ni/l 28 days prior to maing($NiCl_2.6H_2O$)	rel. weight of livers of 100 mg/l females decreased; no other differences in bw, rel weight of liver or kidneys in dams or pups. Ni cumulated in kidney, no alteration of Zn and Cu levels in pup tissues	Decreased fertility index on exposing both males and females or males only 28 days prior to mating (50%) and males 42 days prior to mating (17% decrease). Decreased gestation index after exposure of males for 28 days. No effects on gestation length. Mean diameters of seminiferous tubules were smaller after 28 and 42 days of exposure. After 28 days, fewer basal spermatogonia per 250 µm along the outer edge of the tubules and ratio open/closed tubules increased.	animals differed in age from 2.5-9 months 1 female (100 mg/l) ate all three pups it gave birth to and died soon after that (cause unknown) results difficult to interpret as a result of the small amount of animals in the groups (n=6)
Intratesticular						
Kamboj *et al*. (1964)	male albino rats (n=3), male Swiss mice (n=3)	rats: single injectionfor 2, 7 or 30 daysmice: daily injections for 2, 7 or 30 days	rat: 14.6 mg $Ni(NO_3)_2$/kg bw mice: 14.6 mg $Ni(NO_3)_2$/kg bw sc	Not presented	rats: decrease testicular weight, focal necrosis in testis after 7 daysmice: shrinkage tubules and spermatogenic arrest after 7 days	
Subcutane						
Hoey *et al*. (1966)	male albino rats (n=3-5)	single or daily injections for 30 days	6.2 mg $NiSO_4$/kg	not presented	acute and chronic changes in histology of the testis and interference with spermatogenesis	

Table 1.5 Fertility studies with soluble nickel salts.

authors	species	experimental period/design	dose	general toxicity	effects on reproductive organs/ effects on reproduction	remarks
Intraperitoneal						
Mathur *et al*. (1977a)	male albino rats (n=30)	daily injections during 90 day-ssacrifice after 2, 7, 15, 30, 60 or 90 days	3 mg Ni/kg bw ($NiSO_4$)	mild congestion in liver and kidney after 60 and 90 days. Necrosis in liver, kidney and myocardium at day 90.	increased level of adenosine triphosphatase in testis after 30, 60 and 90 days and decreased level of acid phosphatase after 60 and 90 days. After 90 days degenerative changes in a few seminiferous tubules.	5 animals died during study
Deknudt & Léonard (1982)	male BALB/c mice (n=40)	single injection; mating with untreated females 5x at weekly intervals	0 and 12 mg Ni ($NiCl_2$ or $Ni(NO_3)_2$)	not presented	12 mg $NiCl_2$: decreased incidence of pregnant females w1-4, decreased mean no. of implantations/female12 mg $Ni(NO_3)_2$): decreased incidence of pregnant females w1-5, decreased mean no. of implantations/female	dominant lethal test; 3 animals died after 1 week of treatment ($NiCl_2$, or $Ni(NO_3)_2$)
Xie *et al*. (1995)	male ICR mice (n=5-6)	single injection; 7 days after treatment males were mated with unexposed females for 7 days (females:males 2:1)	0, 0.5, 1.0, 3.0 and 5.0 mg Ni/kg bw ($NiCl_2$)	no data presented	lipid peroxidation, Ni, Ca and Fe concentration in testis were increased dose-dependent 24 h after treatment-testis lipid peroxidation increased 6 h and testicular weight decreased 3 days after treatment with 5 mg Ni/kg bwfertility rate decreased to 80 and 50% after treatment with 1 and 5 mg Ni/kg bw, respectively	

w = weeks; bw = body weight; d = day; inh= inhalation, ip= intraperitoneal, sc = subcutane, LDH = lactate dehydrogenase

Table 1.6 Fertility studies with insoluble nickel salts: Inhalation.

authors	species	experimental period/design	dose	general toxicity	effects on reproductive organs/ effects on reproduction	rem.
Benson *et al* 1987	male and female F344/ N rats (n=6/ group)	12 days, 6h/d, 5 d/w	0, 0.6, 1.2, 2.5, 5.0, 10.0 mg Ni_3S_2/m^3	10 mg/m^3: 2 male rats died .5and 10 mg/m^3: emphysema 0.6 mg/m^3: degeneration of nasal epithelium	5 and 10 mg/m^3: testicular degeneration	
	male and female B6C3F1 mice (n=6/ group)	12 days, 6h/d, 5 d/w	0, 0.6, 1.2, 2.5, 5.0, 10.0 mg Ni_3S_2/m^3	10 mg/m^3: all mice died. 5 mg/m^3: fibrosis 1.2 mg/m^3: degeneration of nasal epithelium	5 and 10 mg/m^3: testicular degeneration	
NTP 1996 (b)	Male and female F344/ N rats (N=5/ group)	16 days:12 days exposure, 6 h/d, 5 d/w	0, 1.2, 2.5, 5, 10, 30 mg nickel oxide/ m^3	No mortality; respiratory toxicity	No effects on sperm number and histology of testes	
		13 weeks 6 h/d, 5 d/w	0, 0.6, 1.2, 2.5, 5, 10 mg nickel oxide/ m^3	No mortality, respiratory toxicity	No effects on sperm number and histology of testes, estrous cycle length 10 mg/m^3: decreased epididymal spermatozoal concentration	
		2 years, 6 h/d, 5 d/w	0, 0.62, 1.25, 2.5 mg nickel oxide/ m^3	No mortality; respiratory toxicity	no effects	
	Male and female B6C3F1 mice (N=5/group)	16 days:12 days exposure, 6 h/d, 5 d/w	0, 1.2, 2.5, 5, 10, 30 mg nickel oxide/ m^3	No mortality; respiratory toxicity	No effects on sperm number and histology of testes	
		13 weeks 6 h/d, 5 d/w	0, 0.6, 1.2, 2.5, 5, 10 mg nickel oxide/ m^3	No mortality, respiratory toxicity	No effects on sperm number and histology of testes, estrous cycle length	
		2 years, 6 h/d, 5 d/w	0, 0.62, 1.25, 2.5 mg nickel oxide/ m^3	No mortality; respiratory toxicity	no effects	
NTP 1996 (c)	Male and female F344/ N rats (N=5/ group)	16 days:12 days exposure, 6 h/d, 5 d/w	0, 0,6 1.2, 2.5, 5, 10 mg nickel subsulfide/ m^3	No mortality; respiratory toxicity	No effects on sperm number and histology of testes	
		13 weeks 6 h/d, 5 d/w	0, 0.15, 0.6, 2.5 mg nickel subsulfide/ m^3	No mortality, respiratory toxicity	No effects on sperm number and histology of testes, estrous cycle length	
		2 years, 6 h/d, 5 d/w	0, 0.15, 1 mg nickel subsulfide/ m^3	No mortality; respiratory toxicity	no effects	

Male and female B6C3F1 mice (N=5/group)	16 days:12 days exposure, 6 h/d, 5 d/w	0, 0.6, 1.2, 2.5, 5, 10 mg nickel subsulfide/ m^3	No mortality; respiratory toxicity	No effects on sperm number and histology of testes
	13 weeks 6 h/d, 5 d/w	0, 0.15, 0.6, 2.5 mg nickel subsulfide/ m^3	No mortality, respiratory toxicity	No effects on sperm number and histology of testes, estrous cycle length
	2 years, 6 h/d, 5 d/w	0, 0.6, 1.2 mg nickel subsulfide/ m^3	No mortality; respiratory toxicity	no effects

Table 2.1 Developmental toxicity studies with nickel carbonyl.

authors	species	experimental period/design	dose	materal toxicity	effects on reproductive organs/ effects on reproduction	rem.
Inhalatory						
Sunderman *et al*. (1978a)	female Fischer rats (n=6-7)	Single exposure GD 8, sacrifice GD 20	60 or 120 mg $Ni(CO_4)/m^3$ for 15 min	no effects presented	120 mg: decreased no. of live foetuses per dam and foetus bw, increased ratio death foetuses to conceptuses 60 and 120 mg: increased no. of anophthalmia and microphthalmia	abstract
Sunderman *et al*. (1979)	female F344 rats (n=12-19)	single exposure GD 7, 8 or 9, sacrifice GD 20	GD 7: 160 or 300 mg GD 8: 80 or 160 mg GD 9: 160 mg $Ni(CO)_4$ / m^3 for 15 min	GD 7: 160 mg no maternal mortility; toxicity not presented GD 7: 300 mg 47% of dams died before GD 20 GD 8: 80 mg no maternal mortility; toxicity not presented GD 8: 160 mg 13% of dams died before GD 20GD 9: no maternal mortality; toxicity not presented	GD 7: both groups increased no. of foetuses with anophthalmia and microphthalmia GD 8: 80 mg no foetal toxicity GD 8: 160 mg increased no. of foetuses with microphthalmia GD 9: no foetuses with malformations	
Sunderman *et al*. (1979)	female F344 rats (n=9)	single exposure GD 7, sacrifice PN wk 16	0 and 300 mg $Ni(CO)_4$ /m^3 for 15 min	maternal toxicity not presented	increased no. of abnormalities (anophthalmia, microphthalmia), decreased pup weight wk 4 and 16	
Sunderman *et al*. (1980)	female Syrian hamsters (n=9-33)	single exposure GD 4, 5, 6, 7 or 8, sacrifice GD 15	60 mg $Ni(CO_4)/m^3$ for 15 min	maternal death on GD 4 (25%) and GD 5-8 (49-59%)	GD 4, 5: increased no. of foetal abnormalities (exencephaly, haemorrhages)	
Sunderman *et al*. (1980)	female Syrian hamsters (n=14)	single exposure GD 5, sacrifice PN 65	60 mg $Ni(CO_4)/m^3$ for 15 min	5 dams died before GD 16	increased neonatal mortality PN 1-4; no behavioural or developmental abnormalities and no congenital malforma- tions or pathological lesions	

Table 3.1 Developmental toxicity studies with soluble nickel salts.

authors	species	experimental period/design	dose	materal toxicity	effects on reproductive organs/ effects on reproduction	rem.
Gavage						
Pandey *et al*. (1999)	see Pandey *et al*. (1999) in Table 1 Fertility studies with soluble nickel salts					
Drinking water						
Smith *et al*. (1993)	female Long-Evans rats (n=34)	females during premating (11w), mating, gestation and lactation, males were unexposed; sacrifice after L2	0, 10, 50 and 250 mg Ni/l ($NiCl_2$) water	no effects observed	10 mg/l: increased no. of dead pups PN1 (L2) 50 mg/l: reduced weight gain of male pups (L1); increased no. of dead pups PN1 (L2) 250 mg/l: dams decreased liquid consumption, more food/ kg bw (prebreeding, G1, G2), reduced weight gain (prebreeding), decreased plasma prolactin levels, increased no. of dead pups PN 1 and 21 (L1 and L2)	
Käkelä *et al*. (1999)	male and female Wistar rats (n= 6)	males, females or both premating, females gestation, lactation	F: 10, 30 or 100 mg Ni/l 14 days or 30 mg Ni/l 100 days prior to mating M: 30 mg Ni/l 28 or 42 days prior to mating F+M: 30 mg Ni/l 28 days prior to mating water ($NiCl_2.6H_2O$)	rel. weight of livers of 100 mg/l females decreased; no other differences in bw, rel weight of liver or kidneys in dams or pups. Ni cumulated in kidney, no alteration of Zn and Cu levels in pup tissues	decreased litter size at weaning (PN21) in a) 100 mg/l female, b) 28 day male and c) both male and female groups. pups of 100 mg/l females with short and sparse hair, dead pups were oedemic.no differences in bw of pups, pups that died during lactation were runts	animals differed in age from 2.5-9 months1 female (100 mg/l) ate all three pups it gave birth to and died soon after that (cause unknown)

Table 3.2 Developmental toxicity studies with soluble nickel salts.

authors	species	experimental period/design	dose	materal toxicity	effects on reproductive organs and reproduction	rem.
Diet						
Ambrose *et al*. (1976)	male and female Wistar rats (n=25)	premating, mating, gestation and lactation during 3-generations	0, 250, 500 and 1000 mg Ni/kg feed ($NiSO_4$)	slightly lower body weights of F0 at 1000 mg/kg	all three feeding levels: F1, increased still born pups1000 mg/kg: F1, F2 and F3 decreased pup weight at weaning-gross observations on pups showed no teratogenic effects	
Intravenous						
Ferm *et al*.(1972)	Syrian hamster (n=2-6)	single injection GD 8,	0.7-10 mg/kg bw ($NiCH_2$-COOH)	no effects presented	dose-related increase in no. of resorptionsseveral unspecified malformations in pups	
Intramuscular						
Sunderman *et al*. (1978b)	female F344 rats (n=11-13)	single injection GD 8, sacrifice GD 20	0, 8, 12 and 16 mg Ni/kg bw ($NiCl_2$)	no effects presented	8 mg: no effects12 mg: reduced no. of live foetuses 16 mg: reduced no. of live foetuses, reduced foetal body weight; no visceral or skeletal anomalies in foetuses	
Sunderman *et al*. (1978b)	female F344 rats (n=7)	single injection on GD 8, sacrifice 8w PN	0 and 16 mg Ni/kg bw ($NiCl_2$)	no maternal toxicity	reduced no. of live pups, decreased pup weight PN w 4-8; no specific behavioural or developmental abnormalities in foetuses	
Sunderman *et al*. (1978b)	female F344 rats (n=12-13)	2 daily doses on GD 6-10, sacrifice GD 20	0, 1.5 and 2 mg Ni/kg bw ($NiCl_2$)	no maternal toxicity	1.5 mg: no effects 2 mg: increased intrauterine death, no skeletal or visceral anomalies in foetuses	
Sunderman *et al*. (1978b)	female F344 rats (n=10-15)	single injection on GD 18, sacrifice GD 19	0, 6, 8 and 16 mg Ni/kg bw ($NiCl_2$)	16 mg: maternal mortality (50%)	6 and 8 mg: no effects 16 mg: increased no. of dead foetuses; no skeletal or visceral anomalies	

Table 3.3 Developmental toxicity studies with soluble nickel salts.

authors	species	experimental period/design	dose	maternal toxicity	developmental toxicity	rem.
Intraperitoneal						
Storeng *et al*. (1981)	Female NMRI/ Bom mice	single injection GD 0, 1, 2, 3, 4 or 5, sacrifice GD 18	20 mg $NiCl_2.6H_2O$/kg bw	no effects presented	GD 0 decrease on average number of implantations per damall days higher resorption frequencies, increase abnormalities (haematomas, exencephaly, anaemic appearance, hypodevelopment) GD 0, 2 and 4 decreased litter size GD 0, 1, 2, 3 and 4 lower average bw of live foetuses	
Mas *et al*. (1985)	female Wistar Porton rats (n=1-6)	single injection on GD 8, 12, or 16, sacrifice GD 20	0, 1, 2 and 4 mg Ni/kg bw ($NiCl_2$)	no effects presented	2 mg (GD 8): increased no. of malformed foetuses (haemorrhages) 4 mg (GD 8): increased no. of malformed foetuses (hydrocephalus, haemorrhages, hydronephrosis) 1, 2, 4 mg (GD 12): increased no. of malformed foetuses (hydrocephalus, haemorrhages, hydronephrosis) 2, 4 mg (GD 12) reduced foetal weight	number of pregnant females very small,1-6/ group1, 2, 4 mg (GD 16): no effects

Table 3.4 Developmental toxicity studies with soluble nickel salts.

authors	species	experimental period/ design	dose	maternal toxicity	developmental toxicity	rem.
Intraperitoneal						
Lu *et al*. (1979)	female CD-1 mice (n=7-10)	single injection GD 7, 8, 9, 10 or 11, sacrifice GD 18	0, 1.2, 2.3, 3.5, 4.6, 5.7 and 6.9 mg Ni/kg bw ($NiCl_2$)	GD 7 and 8: 1 dam died at 6.9 mg/kg; GD 9, 10 and 11: several dams died at 4.6 mg/kg	1.2 mg (all GDs): no effects 2.3 mg (GD 11): increased % foetal death 3.5 mg (GD 7): increased % foetal death 3.5 mg (GD 8, 9, 10, 11): increased % foetal death, decreased foetal weight, increased foetal abnormalities 4.6 mg (GD 7, 8, 9, 10, 11): increased % foetal death, decreased foetal weight, increased foetal abnormalities 5.7 mg (GD 7, 8, 9): increased % foetal death, decreased foetal weight, increased foetal abnormalities 5.7 mg (GD 10, 11): foetal death 100 % 6.9 mg (GD 7, 8): increased % foetal death, decreased foetal weight, increased foetal abnormalities 6.9 mg (GD 9, 10,11): foetal death 100 %	effects were dose related
Diwan *et al*. (1992)	female F344/ NCR rats (n=14-17)	(1) single injection GD 16 (2) two injections GD 15 and 17 offspring water (1A and 2A) or Na barbital (1B and 2B) wk 4-85(3) four injections GD 11, 13, 15 and 17	(1) 17.1 mg/ kg bw (2, 3) 8.5 mg/kg bw $NiCH_2COOH.4H_2O$	(3) offspring died within 72 h after birth no effects presented on dams	body weight of male offspring in all treatment groups lower at 75 wk higher tumour incidence in offspring of 1A and 2A treatment groupshigher incidence of renal cortical epithelial and renal pelvic transitional epithelial tumours in male offspring of 1B and 2B	

Table 4.1 Developmental toxicity studies with insoluble nickel salts.

authors	species	experimental period/design	dose	maternal toxicity	developmental toxicity	rem.
Inhalatory						
Weischer *et al*. (1980)	female Wistar rats (TNO W74) (n=10-13)	continuously exposure GD 0-21, sacrifice GD 21	0, 0.8, 1.6 or 3.2 mg/m^3 NiO	reduced bw gain of dams of all groups 1.6 and 3.2 mg/m^3: wet weight kidneys and no. of erythrocytes decreased, haematocrit and MVC increased 0.8 mg/m^3: no. of leukocytes increased 3.2 mg/m^3: concentration urea in serum decreasedno change in haemoglobin, alkaline phosphatase in serum and urine, bilirubin, urea in urine and wet weight of livers	1.6 and 3.2 mg/m^3: foetal bw reduced 1.6 mg/m^3: no. of leukocytes and urea in serum of foetuses enhanced no changes in no. of foetuses and placentas, wet weight of placentas, haemoglobin, haematocrit, erythrocytes, MVC and alkaline phosphatase in serum of foetuses	
Intramuscular						
Sunderman *et al*. (1978b)	female F344 rats (n=12)	single injection on GD 6; sacrifice GD 20	0 and 80 mg Ni/kg bw (Ni_3S_2)	no maternal mortality within 2 weeks after treatment, no maternal toxicity presented	reduced mean number of live foetuses per damno skeletal or visceral anomalies in foetuses	transplacental carcinogenesis test
Sunderman *et al*. (1981)	female F344 rats (n=8)	single injection GD 6, litter	20 mg Ni_3S_2	no effects presented	decreased body weights of pups, no differences in number of pups, incidence of tumour or non-neoplastic lesions and cumulative mortality rate	

Table 5.1 Studies with soluble nickel salts on lactation.

authors	species	experimental period/design	dose and route	maternal toxicity	developmental toxicity	rem.
Dostal *et al*. (1989)	female Sprague-Dawley (CD) rats (n=6-7)	multiple doses during lactation	6.5 or 13.0 mg $NiCl_2$/kg bw PN 12-15 sc	no effects on maternal bw, liver weight or hepatic lipid oxidation. 6.5 and 13 mg: decreased food consumption 13 mg: reduced thymus weight	altered milk composition (milk solids and lipid increased, lactose decreased)reduced milk synthetic activity milk Ni concentrations 513 and 1030 µg/l, resp.Ni plasma concentrations in pups 24 and 50 µg/l, resp.13 mg: pup liver weights decreased	

Annex **E**

Calculation safe levels of nickel in (human) breast milk

Assumptions

Body weight woman: 60 kg
Body weight infant: 4.5 kg (4-5 kg)
Intake breast milk: 900 ml (800-1000 ml)
An infant is as sensitive for the effects of nickel as an adult.

Calculation of the safe level of nickel in (human) breast milk

The RIVM (Jan94) proposed an ADI for nickel of 5 µg/kg bw/day.
Safe intake level per infant is 22.5 µg/infant/day.
Safe level of nickel in breast milk is 25 µg/l.

In conclusion, the committee considers 25 µg Ni/l breast milk as a safe level.

Annex **F**

Abbreviations

Abbreviations used:

bw	body weight
CI	confidence interval
CNS	central nervous system
d	day
F	female(s)
GD	gestation day
i.p.	intraperitoneal
IRPC	increased renal pelvic cavitation
i.v.	intravenous
M	male(s)
n	number
NOAEL	no adverse effect level
OECD	Organisation for Economic Cooperation and Development
OR	Odds ratio
OT	Operating theatre
PN	postnatal
RR	relative risk