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Nickel, inorganic and soluble salts

Evaluation of health hazards and proposal of a health based quality criterion for drinking water

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Nickel, inorganic and soluble salts. Evaluation of health hazards and proposal of a health based quality criterion for drinking water

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Content

Preface

Nickel metal and four nickel salts (nickel sulphate, nickel chloride, nickel dinitrate and nickel carbonate) are high production volume (HPV) chemicals prioritised for risk assessment within the EU Existing Substances Regulation. The Danish Environmental Protection Agency as a Rapporteur for the five nickel substances has prepared a comprehensive risk assessment report (RAR) for each of these substances. In addition, a background report has been prepared by the Rapporteur including general information about nickel that is common to all the individual reports. The Department of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, has prepared the chapter on human health effects (chapter 4) in the RARs.

A large amount of information was provided by industry for the compilation of the data presented in the RARs. The additional data on nickel from published literature have been reviewed in good quality reviews including UK HSE (1987), IARC (1990), IPCS (1991, 1996), ATSDR (1995) and a Nordic Expert Group (Aitio 1995). The effects of nickel on the skin have also been reviewed (Maibach & Menné, eds. 1989, Hostýnek and Maibach 2002). NiPERA in collaboration with Eurométaux have also produced a criteria document for nickel and nickel compounds for the European Commission (NiPERA 1996). Toxicology Excellence for Risk Assessment (TERA) has prepared a toxicological review of soluble nickel salts for Metal Finishing Association of Southern California Inc., US-EPA and Health Canada (TERA 1999).

These reviews plus (where considered relevant) the primary literature, have been used widely in assembling the RARs as it was determined that much of the essential data to establish possible hazards and risks of nickel for human health has already been adequately evaluated.

This document has been prepared mainly on the basis of the final version of the background report dated March 2008 (RAR background 2008) and thus, all the information in this document has been extracted from this report unless otherwise stated. In sections 4.4, 4.5 and 4.7, also the final versions of the RARs on the individual soluble inorganic nickel salts (nickel sulphate, nickel chloride and nickel dinitrate) dated May 2009 (RAR sulphate 2009, RAR chloride 2009, RAR nitrate 2009) have been consulted. In addition, the final version of the report “Humans exposed indirectly via the environment and combined exposure – exposure assessment and risk characterization” dated April 2008 (RAR MvE 2008) has been consulted regarding sections 1.3 and 1.4. Furthermore, the final version of the nickel metal RAR (RAR metal 2009) has been consulted for some specific information on the metal. No further references have been consulted specifically in the preparation of this document.

This report has been prepared by Department of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The document has been elaborated according to the overall practice laid down in the Danish EPA guideline document for the setting of health based quality criteria for chemical substances in relation to soil, ambient air and drinking water (Vejledning fra Miljøstyrelsen 5/2006).

Furthermore, the document has been subjected to review and discussion and has been adopted in a steering committee with representatives from the following Danish authorities/ institutions:

- The National Board of Health

- The Danish Nature Agency

- The Danish Veterinary and Food Administration

- Danish Regions

- Danish Environmental Protection Agency

- Faculty of Agricultural Science, Aarhus University

1 General description

Nickel is a silver metal with typical metallic properties. It occurs in the periodic table in the first triad of Group VIII after iron and cobalt, to which it is closely related. (RAR metal 2009).
Nickel can be found in a variety of oxidation states ranging from 0 to IV. However, Ni(II) is the only oxidation state occurring in ordinary chemistry. Ni(III) and Ni(IV) occur in certain complexes and in specific oxide systems, the higher oxidation states, however, being considerably less stable than Ni(II). Ni(0) and Ni(I) compounds are scarce. (RAR background 2008).
Ni(II) forms a wide variety of compounds ranging from simple inorganic complexes (salts) to complexes with various organic ligands. It appears that in the aqueous chemistry Ni(II) is the only oxidation state that has to be considered. In the absence of strong complexing agents Ni(II) appears in aqueous solution as the green hexaquonickel (II) ion Ni $(H_2O)_6^{2+}$. (RAR background 2008).

All nickel compounds as well as metallic nickel release the biologically active form, the nickel ion (Ni^{2+}), in the environment and in the tissues of organisms. Therefore, toxicological effects can be considered together for these substances to the extent that these effects are directly mediated by the nickel ion. It is assumed that the nickel ion is the determining factor for systemic toxicity. Solubility is considered as being particularly important for the release of the nickel ion and thus, the systemic bioavailability of the nickel ion. Ideally, data on the solubility of the nickel compounds in biological fluids are preferable; however, no data are available regarding the solubility of any of the five prioritised nickel compounds in biological fluids. Therefore, the water solubility will be used as a prediction of the solubility in biological fluids although realising that such a prediction might not be correct as some data indicate that compounds insoluble or slightly soluble in water might be more soluble in biological fluids. It should be noted that with respect to local effects, the nickel ion may not be responsible for the toxic effects in all situations. (RAR background 2008).

This evaluation is limited to consider the toxicity of the nickel ion, and thus nickel salts from which the nickel ion can be liberated, as this form is the relevant one in relation to estimation of a health based quality criterion in drinking water. Only information of relevance for the estimation of a health based quality criterion in drinking water for soluble inorganic nickel salts has been considered and included in this evaluation.

The term “nickel” is used in a generic sense and refers to nickel in general except when specific nickel salts are mentioned. For the purpose of comparison, concentrations and dose levels of the various nickel salts are expressed in terms of nickel equivalents (Ni) whenever possible.

1.1 Identity and physico-chemical properties

The identity and physico-chemical properties of nickel metal and selected soluble inorganic nickel salts are presented in Table 1.

Table 1. Identity and physico-chemical properties of selected soluble inorganic nickel salts)

Nickel salt	CAS-no.	Structural formula	Molecular weight	Physical State	Melting point (°C)	Boiling Point (°C)	Density (g/cm^3)	Water Solubility
Nickel metal	7440-02-0	Ni	58.71	Solid	1455	2730	8.90	Not applicable
Nickel sulphate (anhydrous)	7786-81-4	$NiSO_4$	154.75	Solid	848	-	3.68	293 g/l at 20°C
Nickel sulphate (hexahydrate)		$NiSO_4$, 6 H_2O	262.84	Solid	53.3	-	2.07	625 g/l at 20°C
Nickel sulphate (heptahydrate)		$NiSO_4$, 7 H_2O	280.85	Solid	99	-	1.95	756 g/l at 20°C
Nickel chloride (anhydrous)	7718-54-9	$NiCl_2$	129.60	Solid	973	1001	3.55	642 g/l at 20°C
Nickel chloride (hexahydrate)		$NiCl_2$, 6 H_2O	237.70	Solid	-	-	1.92	2540 g/l at 20°C
Nickel nitrate (hexahydrate)	13138-45-9	$Ni(NO_3)_2$, 6 H_2O	290.79	Solid	56.7	136.7	2.05	2385 g/l at 0°C

1.2 Production and use

Nickel is mined from nickel-containing minerals occurring in nickel deposits of which only the minerals pentlandite, garnierite and nickeliferous limonite are of economic importance. Nickel refining processes uses nickel derived from ores and lead to the production of pure nickel metal in a variety of forms as well as the nickel salts.

The major use sectors of **nickel sulphate** and **nickel chloride** are plating (89% and 71% of total use, respectively) and catalyst production (11% and 29% of total use, respectively). A small but unidentified portion of nickel sulphate and nickel chloride is also applied in chemicals production.
Nickel dinitrate is mainly used for the production of catalysts (50-75%) and the manufacturing of Ni-Cd batteries (10-50%) – together these sectors comprise 92.5% of total EU production. An estimated additional 5-10% is used for other applications; including chemical pre-treatment of products.

1.3 Environmental occurrence and fate

Nickel and its compounds are naturally present in the earth's crust. Nickel is the fifth most abundant element by weight and the 24th most abundant element in the earth's crust, with an average concentration estimated to be about 75-80 mg/kg.

Nickel is released to the environment from natural processes as well as from anthropogenic activities.

1.3.1 Air

Nickel releases to the atmosphere occur from natural processes such as windblown dust and volcanic eruption. Estimates for emissions from natural sources range from 8.5 x 10^6 kg/year (1984) to 1800 x 10^6 kg/year (2001). Estimates for emissions of nickel due to intentional production and use of nickel are around 13 x 10^6 kg Ni/year.

Typical nickel concentrations in European air were reported in an EU position paper from 2000 as 0.4-2 ng/m^3 for rural area, 1.4-13 ng/m^3 for urban background and 10-50 ng/m^3 for industrial area. The EUSES modelled nickel air concentration for the regional scale (2.7 ng/m^3) falls within the range of the rural and background areas. (RAR MvE 2008).

An update of nickel concentrations in air was made based on two European reference sources (the EMEP database and the Airbase database). The typical nickel ambient air concentrations derived was 4.5 ng Ni/m^3. (RAR MvE 2008).

1.3.2 Water

Both chemical and physical degradation of rocks and soils, atmospheric deposition of nickel-containing particulates, and discharges of industrial and municipal waste release nickel into ambient waters. The main anthropogenic sources of nickel in water are primary nickel production, metallurgical processes, combustion and incineration of fossil fuels, and chemical and catalyst production.

A large monitoring campaign on groundwater quality is running in Denmark, by the Geological Survey of Denmark and Greenland (GEUS). In this water works groundwater monitoring, 6972 wells have been analysed for nickel in a period from 1993-2002 and in 3362 of the wells, nickel was detected. In 221 wells, the drinking water limit at 20 µg Ni/l was exceeded. The median value was 2 µg Ni/l and the 95^{th} percentile was 8.8 µg Ni/l. The highest detected value was 590 µg Ni/l. (RAR MvE 2008).

Nickel can be released from drinking water installations to the drinking water. The release of nickel from water installations is the result of a complicated process, whereby water meters, fittings and kitchen and bathroom taps (single or mixers) can lead to nickel release into the drinking water. Electrochemical processes between the piping material and the water are leading to corrosion whereby metal is oxidised when reacting with oxygen. The metal, now in ion form, will dissolve and go into the water. The release of nickel decreases with the age of the installation. Nickel release is also correlated to the stagnation time of the water within the water installation. In a Danish study of nickel release from existing water installations (double-grip mixer taps made of chromium plated brass), average concentrations were as high as 190 µg Ni/L after an average stagnation time of 8 hours; the nickel concentration was not determined after short-term stagnation in this experiment. In another Danish study (test-rig experiment), similar installations (double-grip mixer taps made of chromium plated brass) were tested with stagnation periods as low as 30 minutes; here the mean nickel concentration was 40 µg Ni/L. (RAR metal 2009).

Based on an updated EU-wide literature survey and data collected from European water supply companies, an average content of 1.5 µg Ni/l and a 95-percentile content of 3.7 µg Ni/l in drinking-water in Europe has been concluded. The data was obtained from tap-water after having been fully flushed and from the inlet of the water supply system and therefore, considered to reflect the background exposure without contribution from taps and fittings. (RAR MvE 2008).

1.3.3 Soil

The primary anthropogenic sources of nickel to soils are emissions from smelting and refining operations and disposal of sewage sludge or application of sludge as a

fertiliser. Secondary sources include automobile emissions from electric power utilities. Weathering and erosion of geological materials also release nickel into soils.

The 50th percentile value for measured soil concentrations in Europe was 14 mg Ni/kg soil (dry matter 'dm'). However, modelled values were preferred over the measured data because measured data were only available for a few countries, which may be not representative for all of Europe. The EUSES modelled nickel concentrations in soil for the regional scale were 19 mg Ni/kg dm for agricultural soil, 17 mg Ni/kg dm for natural soil and 18 mg Ni/kg dm for industrial soil, respectively. (RAR MvE 2008).

Several studies have reported on the accumulation of trace metals in indoor dust. There was a fairly narrow range in median concentrations of nickel in house dust among different studies (19-71 mg Ni/kg dm). Averaged over these studies, a typical nickel house dust concentration of 49 mg Ni/kg was derived. (RAR MvE 2008).

Results from several studies indicated that the median nickel concentrations in street dust for Europe ranged from 16 to 248 mg/kg dm. Average over 6 of the median values, a typical nickel street dust concentrations of 115 mg Ni/kg was derived. (RAR MvE 2008).

1.3.4 Foodstuffs

Nickel concentrations in foods can vary widely, depending on the food type, conditions and location under which the food products were produced and stored. Rich food sources of nickel are oats, chocolate, breakfast cereals, bread, nuts, dried beans, peas and pods, other grains and some vegetables. Products of animal origin generally contain low nickel concentrations. As a general overview, the ranges in mean concentrations across different studies for 11 main food categories are listed in Table 2. The database consists of nickel contents in foods sold on the EU market, starting from 1984 to 2005. (RAR MvE 2008).

1.3.5 Bioaccumulation

Organisms can accumulate metals from dissolved and dietary pathways. Although nickel does bioaccumulate in aquatic biota, the bioaccumulation is generally low. Bioconcentration factors (BCF) were highest for the marine bivalve, *Cerastoderme edule*. For example, BCFs for *C. edule* were as high as 26500, whereas the highest BCF for any other organism was 5613 as reported for the cyanobacterium *Anacystis nidulans*. In general, BCFs for other bivalves were < 340 (median = 270). This suggests that the BCFs observed for *C. edule* are not representative of other bivalves or aquatic organisms. Bioaccumulation factors (BAF) for earthworms are quite low, with the geometric mean BAF being 0.30. Some animal species seem to be able to regulate the nickel content of their tissues by controlled uptake and excretion / storage. An inverse relationship between the BCF and exposure concentration in water has been observed for certain aquatic organisms within the concentration range 1-100 µg/l. This may support the hypothesis that within these concentration ranges active regulation of the uptake of nickel may take place. (RAR ENV 2008).

Table 2. Mean nickel concentration ranges in food items across different EU studies (RAR MvE 2008)

Food categories	Food items	Mean concentration range mg Ni/kg fresh weight
Meat		
	General	<0.014-0.12
	Chorizo and salami	0.21-0.87
Fish		<0.01-0.23
Milk and milk products		
	Milk	<0.01-0.20
	Cheese	0.1-1.02
	Yoghurt	0.01-0.26
Eggs		<0.01-0.28
Fruit		
	Fresh fruit	<0.01-0.14
	Canned fruit	0.31
Potatoes and vegetables		
	Potatoes	0.05- .26
	Vegetables	<0.01-0.52
Pods and nuts		
	Pods	0.10-0.33
	Beans	0.15-0.26
	Peas	0.05-0.42
	Nuts	0.17-2.5
Grains and grain products	Bread	0.07-0.56
	Biscuits	0.06-0.18
	Cereals	0.17-0.68
	Oats	0.77-1.76
	Pasta	0.02-0.19
	Flour	0.1-0.63
Edible oils and fats		
	Oils and fats	0.02-0.4
	Butter	0.02-0.1
	Margarine	0.02-0.34
Sugar products		
	Sugar and preserves	0.05-0.42
	Chocolate	0.63
Beverages		
	Soft drinks and mineral water	<0.01-0.03
	Wine	0.02-0.06
	Spirits	0.1
	Beer	<0.01-0.01
	Soup	0.15
	Fruit juices	0.04-0.06

1.4 Human exposure

Human exposure to nickel can result from inhalation of air, consumption of food and drinking water, or incidental ingestion of soil or dust contaminated with nickel. Food is the predominant source of exposure to nickel.

Using the typical nickel ambient air concentration of 4.5 ng Ni/m^3, and assuming the inhalation rate as 0.5 m^3/kg bw/day (for children 1-5 years old), the inhalation exposure to nickel would be 2.3 ng Ni/kg bw/day. For an adult, assuming an average inhalation rate as 13 m^3/day or a high inhalation rate as 20 m^3/day, the daily inhalation exposure to nickel from ambient air would be about 60 ng/day (average, about 0.9 ng Ni/kg bw/day assuming an adult body weight of 70 kg) or about 90 ng/day (high, about 1.3 ng Ni/kg bw/day).

Using the median value for the concentration of nickel in Danish ground water of 2 µg Ni/l, and the consumption rate of 0.03 l/kg bw/day (median value for children 1-10 years old), the intake from drinking water would be 0.06 µg Ni/kg bw/day (assuming no dilution of groundwater). For an adult, assuming an average consumption rate of 1.4 litre/day or a 90th percentile of 2.3 litre/day, the daily exposure to nickel from drinking water would be 2.8 µg/day (average, about 0.04 µg Ni/kg bw/day assuming an adult body weight of 70 kg) or 4.6/day µg (90th percentile, about 0.07 µg Ni/kg bw/day).
Using the 95th percentile for the concentration of nickel in Danish ground water of 8.8 µg Ni/l, and the consumption rate of 0.08 l/kg bw/day (95th percentile for children 1-10 years old), the intake from drinking water would be 0.7 µg Ni/kg bw/day (assuming no dilution of groundwater). For an adult, assuming an average consumption rate of 1.4 litre/day or a 90th percentile of 2.3 litre/day, the daily exposure to nickel from drinking water would be 12.3 µg/day (average, about 0.2 µg Ni/kg bw/day) or 20 µg/day (90th percentile, about 0.3 µg Ni/kg bw/day).

Nickel can also be released from drinking water installations to the drinking water. Thus, apart from being exposed to background concentration in the potable water coming from water works, consumers may be exposed to nickel released from water installations to the drinking water. To construct a reasonable worst-case scenario it is assumed, that a 60 kg person drinks a total of 2 litres of water from a double grip mixer tap made of chromium plated brass. Three times daily this person drinks one glass of water (0.2 litre which approximately corresponds to the volume of water standing in the tap, fittings and valves) which has been standing in the mixer for 8 hours. The remaining 1.4 litre of water is assumed to have been standing in the mixer for only 30 minutes. The reasonable worst-case exposure to nickel from water installations is calculated to be 2.8 µg Ni /kg bw/day. Taking into consideration the very high nickel concentrations measured in the two test-rig experiments, the fact that newer installations release more nickel than older ones, it cannot be excluded that consumers for certain periods of their lives may be exposed to even higher levels of nickel arising form drinking water installations. (RAR metal 2009).

Using the 50th percentile value for measured soil concentrations in Europe of 14 mg Ni/kg soil, and an intake of 0.0001 kg soil/day (median value for children 1-3 years old), the intake from soil would be 0.1 µg Ni/kg bw/day (body weight of 13 kg).
Using a typical nickel house dust concentration of 49 mg Ni/kg, and an intake of 0.0001 kg dust/day (median value for children 1-3 years old), the intake from house dust would be 0.4 µg Ni/kg bw/day (body weight of 13 kg).
Using a typical nickel street dust concentrations of 115 mg Ni/kg, and an intake of 0.0001 kg dust/day (median value for children 1-3 years old), the intake from street dust would be 0.9 µg Ni/kg bw/day (body weight of 13 kg).

The most recent data on nickel dietary intake for adults in different countries are presented in Table 3. Based on these data, a typical dietary intake of 115 µg Ni/day (1.6 µg Ni/kg bw/day assuming a body weight of 70 kg) was derived. (RAR MvE 2008).

Based on the reported 95th percentiles (UK, 1997: 210 µg Ni/day; France: 149 µg Ni/day; Denmark, 1985: 252 µg Ni/Day: Denmark, 1990: 281 µg Ni/day; Denmark, 1995: 252 µg Ni/day; Denmark 2000-2003: 261 µg Ni/day), an average of 239 µg Ni/day (3.4 µg Ni/kg bw/day assuming a body weight of 70 kg) was derived. (RAR MvE 2008).

Table 3. Nickel dietary intake studies (adults) in different EU countries (Table 18 in RAR MvE 2008)

Country	Year of the study	Intake (µg Ni/day)
France	2000	94
Italy	1993	107
Sweden	1987	80
Greece	1990	94
Spain	2003	138
U.K.	1997	130
Denmark	2000-2003	152*
Poland	1990	149
Germany	1996	94
Average		115

* 95-percentile: 261 µg Ni/day; 99-percentile: 352 µg Ni/day

Nickel dietary intake data for young children (1-2 years) are very scarce. The nickel dietary intake for children 1-2 years was estimated based on food consumption patterns of 18 months old toddlers, and combining these food consumption data with nickel concentrations of the corresponding food items. Hereto, for each food product (or group), the median nickel concentration of all data listed in the survey of nickel concentrations in foods was applied. The estimated nickel dietary intake calculated in this way was 63 µg Ni/day (4.8 µg Ni/kg bw/day assuming a body weight of 13 kg for children 1-3 years old) for the typical intake for 1-2 years old children, and 107 µg Ni/day (8.2 µg Ni/kg bw/day assuming a body weight of 13 kg for children 1-3 years old) for a high percentile for 1-2 years old children. (RAR MvE 2008).

Table 4 summarises the exposures from the various media as estimated according to the approach generally applied according to the principles for setting health based quality criteria for chemical substances in ambient air, soil and drinking water .

An overview of the external indirect environmental nickel exposure as estimated in the report “Humans exposed indirectly via the environment and combined exposure – exposure assessment and risk characterization” (RAR MvE 2008) is presented in Appendix 1. For both the regional and local scenarios, dietary exposure seems to be the dominant pathway (> 95 % for the regional scenario and > 75 % for the local scenarios). (RAR MvE 2008).

Table 4. Estimated exposures from various media

Medium	Adults (body weight 70 kg)		Children (1-2/3 years)	
	Average	High exposure	Average	High exposure
Ambient air [a)]	0.9 ng Ni/ kg bw/day	1.3 ng Ni/ kg bw/day	2.3 ng Ni/kg bw/day	-
Drinking water, median level [b)]	0.04 µg Ni/ kg bw/day	0.07 µg Ni/ kg bw/day	0.06 µg Ni/kg bw/day	-
Drinking water, high level [c)]	0.2 µg Ni/ kg bw/day	0.3 µg Ni/ kg bw/day	-	0.7 µg Ni/kg bw/day
Drinking water installations [d)]	2.8 µg Ni /kg bw/day			
Soil	-	-	0.1 µg Ni/kg bw/day	-
House dust	-	-	0.4 µg Ni/kg bw/day	-
Street dust	-	-	0.9 µg Ni/kg bw/day	-
Diet	1.6 µg Ni/ kg bw/day [e)]	3.4 µg Ni/ kg bw/day [f)]	4.8 µg Ni/kg bw/day [e)]	8.2 µg Ni/kg bw/day [g)]

a) Estimations based on a typical nickel ambient air concentration of 4.5 ng Ni/m^3. For adults, average and high exposures are for average (13 m^3/day) and high (13 m^3/day) inhalation rates, respectively.
b) Estimations based on the median value for the concentration of nickel in Danish ground water of 2 µg Ni/l. For adults, average and high exposures are for average (1.4 litre/day) and 90th percentile (2.3 litre/day) consumption rates, respectively.
c) Estimations based on the 95th percentile for the concentration of nickel in Danish ground water of 8.8 µg Ni/l. For adults, average and high exposures are for average (1.4 litre/day) and 90th percentile (2.3 litre/day) consumption rates, respectively.
d) Estimations based on an average nickel concentration of 190 µg Ni/L (average stagnation time of 8 hours) and a mean nickel concentration of 40 µg Ni/L (stagnation time of 30 minutes) and a reasonable worst-case scenario that a 60 kg person drinks a total of 2 litres of water from a double grip mixer tap made of chromium plated brass.
e) Typical dietary intake.
f) An average 95th percentile.
g) High intake.

2 Toxicokinetics

Below, the available data regarding the three prioritised soluble inorganic nickel salts (nickel sulphate, nickel chloride, and nickel nitrate) are summarised. Data on other nickel compounds are also summarised below when considered relevant for the purpose of this evaluation. All information on toxicokinetics is extracted from the background report (RAR background 2008).

2.1 Absorption

Nickel metal and its inorganic compounds can be absorbed in humans and in animals via the gastrointestinal tract as well as the respiratory passages. Percutaneous absorption is negligible quantitatively. The relative amounts of nickel absorbed are determined, not only by the quantities administered, but also by the physical and chemical characteristics of the nickel compound. Solubility is an important factor in all routes of absorption. Soluble nickel compounds dissociate readily in the aqueous environment of biological membranes, thus facilitating their transport as the nickel ion. Conversely, insoluble nickel compounds are relatively poorly absorbed. It is possible that other factors, such as host, nutritional and physiological status, or stage of development, also play a role, but these have not been studied.

2.1.1 Oral intake

Absorption of nickel from the gastrointestinal tract occurs after ingestion of various nickel compounds in food, beverages, or drinking water. The rate of nickel absorption from the gastrointestinal tract is dependent on the chemical form and thus, the solubility. Furthermore, absorption may be suppressed by binding or chelating substances, competitive inhibitors, or redox reagents. On the other hand absorption is often enhanced by substances that increase pH, solubility, or oxidation, or by chelating agents that are actively absorbed. While soluble nickel compounds are better absorbed than relatively insoluble ones, the contribution of the poorly soluble compounds to the total nickel absorption may be more significant, since they are more soluble in the acidic gastric fluids.

Absorption of nickel following oral ingestion of **nickel sulphate** has been evaluated in a number of human studies; however, it is impossible to give a general estimate for the fraction of nickel absorbed after oral administration of nickel sulphate. The available studies indicate that the extent of absorption is influenced by whether nickel sulphate is administered in drinking water, to fasting subjects, or together with food. One study in human volunteers showed that 27% of a dose was absorbed when nickel sulphate was administered in drinking water to fasting subjects compared with around 1% when administered together with food to fasting subjects. Another study supports an absorption of about 1 to 5% when nickel sulphate was administered in lactose. A higher absorption fraction of 4 to 20% was observed after ingestion of nickel sulphate during fasting. In addition, absorption of nickel was apparently slower when administered together with food compared with water.
One study in rats showed an absorption of 11% when nickel sulphate was administered in a 5% starch saline solution.

Absorption of nickel following oral ingestion of **nickel chloride** has been evaluated in a few studies in experimental animals; no human data have been located. When non-fasting rats were dosed by gavage with nickel chloride, 3 to 6% of the nickel was absorbed regardless of the administered dose. Another study in rats showed an absorption of 9.8% when nickel chloride was administered in a 5% starch saline solution. In mice, the intestinal absorption was estimated to be 1.7 to 10% when nickel chloride was administered orally by gastric intubation.

Absorption of nickel following oral ingestion of **nickel nitrate** has been evaluated in one study in rats, which showed an absorption of 34% when nickel nitrate was administered in a 5% starch saline solution. No human data have been located.

The influence of fasting and food intake on the absorption and retention of **nickel** added to drinking water has been examined. Eight non-allergic male volunteers received nickel (compound not specified) in drinking water (12 µg Ni/kg bw) and, at different time intervals, a standardised 1400 kJ portion of scrambled eggs. Before each nickel intake, the volunteers fasted for 12 hours (overnight). When nickel was ingested in water 30 minutes or one hour prior to the meal, peak nickel concentrations in serum occurred one hour after the water intake, and the peak was 13-fold higher than the one seen one hour after simultaneous intake of nickel-containing water and scrambled eggs. In the latter case, a smaller, delayed peak occurred 3 hours after the meal. Within 3 days, the amount of nickel excreted in the urine corresponded to 2.5% of the nickel ingested when it was mixed into the scrambled eggs. Increasing amounts were excreted as the interval between the water and the meal increased, with 25.8% of the administered dose being excreted when the eggs were served 4 hours prior to the nickel-containing drinking water.
In a second experiment, a stable nickel isotope (^{61}Ni, compound not specified) was administered in the drinking water (12 µg Ni/kg bw) to 20 nickel-sensitised women and 20 age-matched controls, both groups having vesicular hand eczema. The course of nickel absorption and excretion in the allergic groups did not differ and was similar to the pattern seen in the first study, although the absorption in the women was less (nickel-sensitised: 10.8%; control: 11.2% - at 72 hours after administration of the dose).
Nickel balance studies have been performed on 10 male volunteers (aged 17 years), who ingested a mean of 289 ± 23 mg Ni/day (range 251-309 mg, compound not specified). Faecal elimination of nickel averaged around 89% (258 ± 23 mg Ni/day).
A biokinetic model has been used to estimate nickel absorption, based on experimental data from various studies. The results showed that estimated nickel absorption ranged from 12-27% of the dose when nickel was ingested after a fast, to 1-6% when nickel was administered either in food, in water, or in a capsule during (or in close proximity to) a meal.

2.1.2 Inhalation

Soluble nickel compounds, such as nickel sulphate, nickel chloride, and nickel nitrate, are expected to be absorbed from the respiratory tract following inhalation exposure. The available data on nickel sulphate and nickel chloride indicate that the absorption of nickel following inhalation of soluble nickel compounds might be as high as up to 97-99%; it should be noted that the fraction absorbed apparently depends on the concentration of the nickel compound in the inhaled air as well as on the duration of exposure. A value of 100% is considered for the absorbed fraction of nickel from the respiratory tract following exposure by inhalation of soluble nickel compounds for particulates with an aerodynamic diameter below 5

µm (respirable fraction). For nickel particulates with aerodynamic diameters above 5 µm (non-respirable fraction), the absorption of nickel from the respiratory tract is considered to be negligible as these particles predominantly will be cleared from the respiratory tract by mucociliary action and translocated into the gastrointestinal tract and absorbed.

Insoluble and slightly soluble nickel compounds, such as nickel metal, nickel carbonate, nickel oxides and nickel sulphides, are expected to be absorbed from the respiratory tract following inhalation exposure to a more limited extent compared to the soluble compounds. The available data on nickel oxide indicate that the absorption of nickel from the respiratory tract following inhalation is very limited. However, data on metallic nickel indicate an absorbed fraction of up to approximately 6% after inhalation (i.e. both absorption from respiratory tract and the gastrointestinal tract).

2.1.3 Dermal contact

The available data indicate that absorption of nickel following dermal contact to various nickel compounds can take place, but to a limited extent with a large part of the applied dose remaining on the skin surface or in the stratum corneum. The data are too limited for an evaluation of the absorbed fraction of nickel following dermal contact.
An *in vitro* study of soluble nickel compounds (nickel sulphate, nickel chloride, nickel nitrate, and nickel acetate) using human skin (Tanojo *et al*. 2001) showed that about 98% of the dose remained in the donor solution, whereas 1% or less was found in the receptor fluid and less than 1% was retained in the stratum corneum. The amount absorbed into the skin, but not passed into the receptor fluid, should also be included in the estimate of dermal absorption and thus, a value of 2% is considered for the absorbed fraction of nickel following dermal contact to soluble nickel compounds.
For nickel metal, a value of 0.2% is considered for the absorbed fraction of nickel following dermal contact based on data from an *in vivo* study in humans (Hostýnek et al. 2001).

2.2 Distribution

Upon entry into the bloodstream, the nickel ion is bound to specific serum components and rapidly distributed throughout the body. In serum, nickel is present in three forms: 1) as a complex associated with albumin; 2) as a complex associated with a nickel-metalloprotein (nickeloplasmin); and 3) as ultrafiltrable material. The most important nickel-binding protein in serum is albumin, which has a high binding capacity for nickel in most species tested, including humans, rats, rabbits, and cows, while the nickel-binding capacity of albumin from dogs and pigs is much lower. The predominant low molecular weight form of nickel in serum, including human serum, is a complex of nickel with the amino acid L-histidine, which play an important role in extracellular transport and in the elimination of nickel in urine. In human serum, 40% of the nickel is present as ultrafiltrable material, 34% is associated with albumin, and 26% is associated with nickeloplasmin.
The predominant intracellular form of nickel has been observed to vary among tissues. In the lung and liver of mice, nickel was bound predominantly to a high-molecular-weight protein; in the kidney, it was bound mainly to low-molecular-weight ultrafiltrable ligands.

Two drinking water studies in rats with **nickel sulphate** have reported some differences in tissue distribution. In one study (6 months, (100 mg Ni/l), the highest concentrations of nickel were found in the liver followed by the kidney. In the other study (13 weeks, about 45-225 mg Ni/l) in male rats, the concentration in different organs was increased with increasing concentrations of nickel in the drinking water; the relative order of bioaccumulation of nickel (at 225 mg Ni/l) was kidneys > testes > lung = brain > spleen > heart = liver. In mice, nickel levels were higher in kidney than in liver. No human data have been located.

Following oral administration by gavage to mice of **nickel chloride** to rats, the highest concentrations of nickel were found in the lungs, kidneys, and liver. No human data have been located.

No studies regarding distribution of nickel in humans or in experimental animals following exposure to **nickel nitrate** have been located.

Nickel has been shown to cross the human placenta. Transplacental transfer has also been demonstrated in mice following administration of nickel chloride by intraperitoneal injection.

Nickel has been found in breast milk of women and in milk from lactating rats administered nickel chloride by subcutaneous injection.

2.3 Elimination

In humans, absorbed nickel is predominantly excreted in the urine following oral intake of **nickel sulphate** with 20 to 30% of a dose being excreted in the urine when nickel sulphate is administered in drinking water to fasting subjects or to fasting subjects compared with around 1 to 5% when administered together with food.
No data on excretion of nickel sulphate in experimental animals following oral administration have been located.

No studies regarding elimination in humans or in experimental animals following exposure to **nickel chloride** or **nickel nitrate** have been located.

3 Human toxicity

All information on human toxicity is extracted from the background report (RAR background 2008).

3.1 Single dose toxicity

In the 19th century, nickel salts were used medicinally, and 325 mg nickel sulphate in solution or in pill form produced nausea and giddiness.

3.2 Irritation

Human data indicate that nickel sulphate in concentrations above 20% can induce skin irritation. Nickel chloride is also an irritant in humans, and is more irritating to the skin than nickel sulphate at equimolar concentrations.

No human data have been found regarding irritation to eyes and the respiratory tract.

3.3 Sensitisation

3.3.1 Skin sensitisation

Nickel is well known as a skin sensitiser, and is one of the most frequent skin sensitisers in man. The nickel ion is considered exclusively responsible for the immunological effects of nickel. Most cases of primary nickel sensitisation are caused by skin contact with metallic items such as ear ornaments, ear stickers, jewellery, jeans buttons, and other nickel releasing items. Solutions of soluble nickel salts may also induce sensitisation, e.g. nickel sulphate in the nickel-plating industry.

The mechanism for development of nickel allergy includes two steps, induction (also called sensitisation) and elicitation. Nickel allergy is induced by direct and prolonged skin exposure to elemental nickel, which is corroded (release of ions) by contact with sweat or by skin exposure to other nickel compounds where nickel ions penetrate into the skin. In order to induce an allergic response, the nickel ion as a hapten must react with a protein in the skin to form a complete allergen, which is then taken up by a macrophage for antigen presentation to a T-lymphocyte. The Langerhans cells of the skin are believed to be responsible for transporting the allergen to the T-lymphocytes in the peripheral lymph node, where antigen presentation takes place. Here, the nickel will be presented to naive T-lymphocytes, and a sensitisation specific to nickel will take place, resulting in clones of specific sensitive memory- and effector-T-cells. This process lasts about 14 days. The next time the individual is exposed to nickel, the specific sensitised T-lymphocytes will elicit an inflammatory response in the epidermis (elicitation) at the site of exposure and possibly elsewhere.

Systemic exposure to nickel orally in individuals without contact allergy to nickel does not result in sensitisation but may result in immunological tolerance, meaning

that the individual is unable to develop contact allergy to nickel at subsequent exposures.

In order to investigate whether oral administration of nickel sulphate were able to worsen hand eczema 5.6 mg nickel as nickel sulphate was given orally to nickel allergic patients with hand eczema in a double-blind investigation; worsening of hand eczema was observed. Two other studies have shown flare of dermatitis after a single oral dose of 0.6 mg nickel and one study after two weekly doses of 0.5 mg nickel. When 12 µg Ni/kg bw (equivalent to 720 µg/60 kg person) was given on an empty stomach to 20 nickel sensitised women and 20 age-matched controls, both groups having vesicular hand eczema, nine of the 20 nickel allergic patients had a worsening of their hand eczema after the nickel administration, and three also developed maculopapular exanthema; no exacerbation was seen in the control group.

3.3.2 Respiratory sensitisation

Five single cases of work related asthma due to exposure to **nickel sulphate** in electro- or metal plating have been reported. In all five cases, the diagnosis was based on clinical picture and specific bronchial inhalation test with nickel sulphate.

For **nickel chloride** and **nickel nitrate** no data regarding respiratory sensitisation in humans have been located.

3.4 Repeated dose toxicity

Human data on repeated dose toxicity of relevance for setting a health based quality criterion in drinking water for soluble inorganic nickel salts have not been located.

3.5 Toxicity to reproduction

A cross sectional study of female nickel hydrometallurgy workers in a Russian refinery plant suggesting increased incidences of spontaneous abortions and malformations during exposure to soluble nickel exposure levels around 0.2 mg/m^3 is considered as inconclusive due to flaws in the study design and reporting. A subsequent study investigated genital malformations in newborns of female nickel-refinery workers using a register-based cohort study design. No negative effects on genital malformations was seen, but, as was also stated by the authors, this result should be interpreted with caution since there were few cases in the higher exposure groups.

No relevant studies regarding effects on fertility have been found.

3.6 Mutagenic and genotoxic effects

No human *in vivo* studies have been located. Tests with human cells *in vitro* are presented in section 4.6.1.

3.7 Carcinogenic effects

No human data regarding carcinogenicity of nickel compounds following oral ingestion have been located.

Epidemiological studies have revealed that nickel compounds are respiratory tract carcinogens in humans following inhalation.
At their meeting in April 2004, the EU Specialised Experts concluded that nickel sulphate and nickel chloride should be considered as human carcinogens. In drawing this conclusion, it was recognised that the epidemiological data showed a clear exposure response relationship for water soluble compounds, consistency across and within studies and time periods, and high strength of association. Improved exposure characterisation based on personal air sampling and improved analysis of the water soluble fractions added to the reliability of the findings. Confounding factors such as co-exposure to insoluble nickel compounds and smoking were adequately addressed, and did not lower the level of confidence in reaching the conclusion. The Specialised Experts also agreed that the 2 other prioritised nickel compounds, i.e., nickel nitrate and nickel carbonate should be classified as human carcinogens. In reaching this conclusion for nickel nitrate the Specialised Experts recognised that the water solubility of this compound was sufficiently similar to that of nickel sulphate and nickel chloride to justify the same classification. Since both the water soluble nickel compounds considered at this meeting and the insoluble inorganic nickel compounds already classified in Annex I are considered as human carcinogens consequently also the nickel carbonate was considered to be a human carcinogen. The TC C&L has agreed to classify nickel sulphate, nickel chloride, nickel nitrate and the nickel carbonates as Carc. Cat. 1; R49.

4 Animal toxicity

All information on animal toxicity is extracted from the background report (RAR background 2008) unless otherwise stated.

4.1 Single dose toxicity

Information on the LD_{50} values of the three prioritised soluble inorganic nickel salts (nickel sulphate, nickel chloride, and nickel nitrate) are summarised in Table 5.

Table 5. LD_{50} values in rats

	Sex	LD_{50}, mg/kg (mg Ni/kg)
Nickel chloride	male female	430 (105) 529 (130)
Nickel chloride hexahydrate	male female	210 (51) 175 (43)
Nickel nitrate		1620 (330)
Nickel sulphate		500 (190)
Nickel sulphate hexahydrate	male female	325 (73) 275 (61)

A toxic class method study showed no mortality or signs of toxic symptoms at 200 mg nickel nitrate hexahydrate/kg.

4.2 Irritation

Nickel metal did not cause skin irritation in an EU Annex V skin irritation test.
Nickel sulphate gave slight irritation (erythema) in an Annex V skin irritation test; in two other studies, the application was repeated for 30 days, and resulted in adverse effects on the skin.
Nickel nitrate hexahydrate was irritant in an Annex V test (as well as a second sample and a solution).

Nickel sulphate gave slight eye irritation (slight degree of conjunctival redness and oedema, and iris lesions) in an EU Annex V eye irritation test.
Nickel nitrate hexahydrate is a severe eye irritant in an Annex V test, as irritation persisted at the end of a 21-day observation period.

No studies examining the respiratory irritation caused by a single exposure to nickel or nickel compounds have been found. Several studies have shown lung inflammation and degeneration of the olfactory epithelium following relatively short periods of exposure to the very soluble nickel sulphate.

Nickel sulphate induced atrophy of the olfactory epithelium and lung inflammation in mice and rats after only 16 days inhalation exposure. The lowest dose tested was equivalent to 0.7 mg Ni/m^3 and this was a LOAEC.
In similar 16-day studies, the insoluble nickel compounds nickel oxide and nickel subsulphide were also shown to cause respiratory effects. Nickel subsulphide was more potent than oxide, and caused lung inflammation and atrophy of the nasal olfactory epithelium in all exposed groups with a LOAEC of 0.44 mg Ni/m^3.
Based on these data it is not possible to relate the type or severity of respiratory effect to solubility characteristics of the nickel compounds.

4.3 Sensitisation

A number of studies on skin sensitisation using different protocols showed that **nickel sulphate** is a skin sensitiser in guinea pigs and mice.

Two studies on skin sensitisation in guinea pigs using various methods showed that **nickel chloride** is a skin sensitiser.

No animal data on skin sensitisation with **nickel nitrate** are available.

No data regarding respiratory sensitisation in animals have been located.

4.4 Repeated dose toxicity

The information on repeated dose toxicity of nickel sulphate and nickel chloride of relevance for the estimation of a health based quality criterion in drinking water for soluble inorganic nickel salts is summarised in Table 6 and further addressed in the text below the table. No data for nickel nitrate have been located.

4.4.1 Nickel sulphate

The following information has been extracted from the individual risk assessment report on nickel sulphate (RAR sulphate 2009). Table 6 gives an overview of the relevant repeated dose oral studies.

4.4.1.1 Rats

A 90-day oral gavage study in rats using nickel sulphate hexahydrate has been performed as a range-finding study for a 2-year carcinogenicity study. The study was performed according to GLP. Groups of 10 male and 10 female rats were given 0, 11, 17, 22, 28, and 33 mg Ni/kg bw/day (0, 50, 75, 100, 125, and 150 mg/kg bw/day of nickel sulphate hexahydrate). Because of significant weight loss in males at the high doses early in the study, the two highest doses were reduced to 30 and 15 mg/kg bw/day, respectively, on day 28 for males only.
One high-dose female rat was found dead on study day 44, the cause of death could not be established.
Clinical observations included post-dosing salivation and decreased activity, most pronounced during the first two weeks and in the highest dose groups.
A variety of statistically decreased absolute or increased relative organ weights were noted in the treated rats. These effects were not accompanied by histopathological changes. The only significant adverse effects seen in this study were weight loss in all dosed groups (8-13% lower body weight compared to

controls). No notable macroscopic or microscopic changes were observed. There was no dose level without effect on body weight, and the **LOAEL** was thus 7 mg Ni/kg bw/day (30 mg/kg bw/day as nickel sulphate hexahydrate (reduced from 125 mg/kg bw/day at day 28)) for males and 11 mg Ni/kg bw/day (50 mg/kg bw/day as nickel sulphate hexahydrate) for females (SLI, draft not dated, submitted 2002).

Table 6. Repeated dose oral studies with nickel sulphate and nickel chloride

Duration and species	Dose levels mg Ni/kg bw/day	NOAEL mg Ni/kg bw/day	LOAEL mg Ni/kg bw/day	Effect at LOAEL	Reference
Nickel sulphate					
13 weeks, rats, drinking water	0, 4.5, 11.2, or 22.4	4.5	11	4% reduction in body weight	Obone *et al*. (1999)
90 days, rats gavage	0, (7)11, 17, 22, 28, and 33	No dose level without effect	(7) 11	8% reduction in body weight	SLI draft not dated (submitted 2002)
3 or 6 months, rats, drinking water	0 or 6.9 (males) or 7.6 (females)	No dose level without effect	7.6	Increased urinary albumin	Vyskocil *et al*. (1994b)
6 months, mice, drinking water	0, 25, 64 or 88 (assuming hexahydrate was given)	No dose level without effect	25	Reduced thymus weight, thymus atrophy	Dieter *et al*. (1988)
2 years, rats, food	0, 10, 100, or 250	10	100	18% reduction in body weight in females	Ambrose *et al*. (1976)
2 years, dogs, food	0, 7.5, 75, or 188	75	188	Decreased body weight gain, lung granulomas, bone marrow hyperplasia	Ambrose *et al*. (1976)
2 years, rats, gavage	0, 2.2, 6.7, or 11	2.2*	6.7	Decreased survival rate, (females) reduced body weight gain (both sexes)	CRL (2005)
Nickel chloride					
13 weeks, rats, gavage	0, 5, 35, or 100	No dose level without effect	5	Death, signs of intoxication	American Biogenics Corporation (1988)

* associated with a slight decrease in body weight gain (both sexes) and survival (females)

Adult male Sprague-Dawley rats (8 animals per group) were given nickel sulphate hexahydrate at concentrations corresponding to 0, 44.7, 111.75, and 223.5 mg Ni/l (0, 0.02, 0.05, and 0.1% nickel sulphate hexahydrate), in their drinking water ad libitum for 13 weeks (Obone et al. 1999).
All animals survived to the end of treatment, and no apparent clinical signs of toxicity were noted. At the highest dosage level a slight, but statistically significant decrease in final mean body weight (4%) was found; body weight was not affected at lower dose levels.
Both the absolute and relative liver weights were decreased (by 15%, estimated from figure by rapporteur) at the two highest dose levels. The relative kidney weight was increased at the low and the highest dose (by 10%, estimated). The absolute lung weight was increased at the lowest and highest dose levels (by 10%,

estimated), while the relative lung weight was increased only at the highest dose level. The absolute, but not the relative, weights of testes and heart were decreased in all groups (by 5%, estimated). The relative weight of the spleen was increased at all dose levels (by 5%, estimated).
There were no gross or microscopic changes in any of the tissues examined at any dose level.
The splenic and thymic lymphocyte subpopulations (T and B cells) were affected in a non-monotonic fashion. In both spleen and thymus, the total number of cells were increased at the middle dose (111.75 mg Ni/l), and reduced at the high dose (223.5 mg Ni/l) compared with control.
The study has several limitations. The group size was only 8, and only males were included. Histopathological evaluation was not performed on all tissues, which normally would be assessed in a repeated dose toxicity study, including tissues relevant for the assessment of effects on the immune system. Nevertheless, the study appears well performed and is considered useful for the evaluation of repeated dose toxicity of nickel sulphate.
The LOAEL is set to 111.75 mg Ni/l based on the 4% reduction in body weight and the increased relative weights of kidney and lungs, which although not considered very serious, are considered as biologically important adverse effect.
The NOAEL is 44.7 mg Ni/l. Although food and water consumption was reported to be measured in the study, no data were given in the publication. Based on an assumed intake of 0.1 litre/kg bw/day, the oral **LOAEL** is 11 mg Ni/kg bw/day (49 mg/kg bw/day as nickel sulphate hexahydrate) and the **NOAEL** is 4.5 mg Ni/kg bw/day (20 mg/kg bw/day as nickel sulphate hexahydrate).

Wistar rats (20/sex/group) were given 0 or 100 ppm nickel as nickel sulphate (hydration state not noted) in drinking water for up to 6 months, with an interim sacrifice of 10/sex/group at 3 months (Vyskocil et al. 1994). Nickel intake was calculated by the authors based on drinking water consumption. Averaged over the two 3-month periods, males consumed 6.9 mg Ni/kg bw/day, and females consumed 7.6 mg Ni/kg bw/day.
There was no effect on body weight gain in either sex.
Kidney weights in both sexes at all time points were slightly higher in the exposed groups, and a slight but statistically significant increase in kidney weight was observed in males at 6 months.
There was no effect on the markers of tubular function. However, urinary albumin levels, a marker of glomerular function, were significantly increased in females at 6 months. Although the increase in urinary albumin in males was not statistically significant, evaluation of the individual animal data showed a clear increase at 6 months; the lack of a statistically significant effect in males was attributable to two control males with abnormally high values. Thus, the single dose in this study, 6.9 mg Ni/kg bw/day in males and 7.6 mg Ni/kg bw/day in females, was a LOAEL for increased urinary albumin.
A limitation of this study is that there was considerable variability in response in both males and females. As part of this assessment, the study authors were contacted in order to obtain the individual animal data and to evaluate the implications of the variability, including a determination of whether individual nickel exposed animals showed increased albuminuria between the 3 and 6-month analyses (baseline values were not obtained). However, the individual data were no longer available. The results in the males are less reliable than those in the females in light of the high degree of variability in urinary albumin levels seen in male rats in general, and because the results in the males were not statistically significant (although they did reflect a population shift). Therefore, the study **LOAEL** is the LOAEL in females of 7.6 mg Ni/kg bw/day for increased urinary albumin.

In a chronic feeding study, groups of 25 male and 25 female weanling Wistar rats were exposed to dietary nickel sulphate hexahydrate in concentrations of 0, 100, 1000, or 2500 ppm Ni (corresponding to 0, 10, 100 or 250 mg Ni/kg bw/day, based on an assumed food intake of 100 g/kg bw/day, Rapporteur´s calculation) (Ambrose et al. 1976). The actual concentrations of nickel sulphate hexahydrate in the diet were not given in the publication, but are calculated to be 0, 45, 450, or 1121 mg/kg bw/day as nickel sulphate hexahydrate. The rats were exposed for up to 2 years. Information on the amount of nickel in the basal diet was not reported. Survival was poor in all groups, including control (68-92% mortality). For this reason, only 2-8 rats per group were available for gross and microscopic pathological examination at sacrifice.
Body weights were significantly lower than control values in females at >1000 ppm from week six and in both sexes at 2500 ppm from the beginning of the study. At 78 weeks, body weights were decreased by 18% in mid-dose females and 8% in mid-dose males; corresponding decreases at the high dose were 32% and 35%.
There were no exposure-related changes in haematology (hemoglobin, hematocrit and differential leukocyte counts) or urinalysis (reducing substances and protein) endpoints.
Relative liver weights were significantly decreased in females at 1000 ppm and relative heart weights were statistically significantly increased in the same group, although there was no clear dose-response.
Histological findings were essentially negative and not indicative of any characteristic effect of nickel in the diet.
This study is limited by the high mortality in all groups, resulting in only a small number of animals being exposed for the total period of 2 years and being available for sacrifice and histopathology.
Based on the 18% decreased body weight in females, which is considered a biologically important adverse effect, a **NOAEL** of 10 mg Ni/kg bw/day (45 mg/kg bw/day as nickel sulphate hexahydrate) and a **LOAEL** of 100 mg Ni/kg bw/day (450/kg bw/day as nickel sulphate hexahydrate) can be determined.

A two-year oral (gavage) OECD TG 451 carcinogenicity study with nickel sulphate hexahydrate in Fischer rats has been conducted (CRL 2005). Groups of 60 male and 60 female rats were dosed with 0, 2.2, 6.7 and 11 mg Ni/kg bw/day (0, 10, 30 and 50 mg/kg bw/day of nickel sulphate hexahydrate) once daily for 104 weeks. The test substance was dissolved in water and given in a gavage volume of 10 ml/kg bw.
Survival of the females was reduced in a dose-related manner and reached the level of statistical significance at the two highest dose levels. The mortality rates for the females at the end of the study were 23%, 33%, 43% and 45% at 0, 2.2, 6.7 and 11 mg Ni/kg bw/d, respectively. There was no apparent treatment-related effect on survival in males (mortality rates 60%, 48%, 50% and 57% at 0, 2.2, 6.7 and 11 mg Ni/kg bw/d, respectively).
In males a statistically significant and dose-related reduced body-weight gain was observed in the dosed groups (5%, 11%, and 12% respectively) compared to the control group. Similarly, in females a dose-related reduced body weight gain (4%, 8% and 10% respectively) was observed when compared to controls but only achieved statistical significance in mid- and high-dose females. The reduced body weight gain had no correlation with the amount of food consumed.
None of the non-neoplastic microscopic findings were considered as being related to dosing with the test substance.
In this study, a **LOAEL** of 6.7 mg Ni/kg bw/day (30 mg/kg bw/day as nickel sulphate hexahydrate) is set based on reduced body weight and increased mortality and a **NOAEL** of 2.2 mg Ni/kg bw/day (10 mg/kg bw/day as nickel sulphate hexahydrate).

4.4.1.2 Mice

A mouse study was conducted in order to determine a threshold response for myelotoxicity and immune-toxicity (Dieter et al. 1988). Groups of 10 female B6C3F1 mice were exposed to graded doses of nickel sulphate (hydration state not reported). The animals were given free access to the chemical in the drinking water at 0, 1, 5 or 10 g/l for 180 days. The measured intake was 0. 115.7, 285.7, and 395.7 mg nickel sulphate/kg bw/day (equivalent to 0, 44, 108 and 150 mg Ni/kg bw/day, or if the reported doses were as nickel sulphate hexahydrate the nickel doses were equivalent to 0, 25, 64, and 88 mg Ni/kg bw/day).
There was no mortality.
Mid- and high-dose mice drank less water than controls; the responses measured in the high-dose group may have been due to a combination of dehydration and chemical toxicity.
Blood nickel was measured at 4, 8, 16, and 23 weeks of exposure. The mean blood nickel values showed increases in the time period between 4 and 8 weeks that were proportional to time and dose. After the 8 weeks there was no substantial increase in blood nickel in any of the dose groups, except for an increase in the mean blood concentration in the 395.7 mg nickel sulphate/kg bw/day dose group at 23 weeks. The kidney was the major organ of nickel accumulation.
Decreases in body and organ weights were confined to high-dose mice, except for the dose-related reductions in thymus weights. Histopathologically, mild tubular nephrosis was observed in all evaluated (6 animals/group) mid and high-dose mice, but not at the low dose or in controls. Mild thymic atrophy, characterised by a decrease in size of the lymphocyte-rich thymic cortex was observed in all treated mice (6/6 in all groups) while minimal atrophy was observed in on1y 1 of 6 control animals. The histology finding of thymic atrophy was supported by statistically significant decreases in thymus weight at all dose levels.
The primary toxic effects of nickel sulphate were expressed in the myeloid system. A significant decrease in the lymphoproliferative response to a B-cell mitogen, but not to a T-cell mitogen, was observed at all doses. In addition, statistically significant decreases in PFC response and spleen cellularity were observed at the high dose. A statistically significant, dose-related decrease in the granulocyte-macrophage proliferative response was observed at all doses, and a decrease in bone marrow cellularity occurred at the two highest doses. Although the decrease in lymphoproliferative response was observed at the low dose, the study authors considered this effect to be secondary to effects on the myeloid system because other immune function parameters were not affected.
Based on the histologically-observed thymic atrophy and decreased thymic weight, the **LOAEL** in this study was 115.7 mg nickel sulphate/kg bw/day (equivalent to 44 mg Ni/kg bw/day, or if the reported doses were as nickel sulphate hexahydrate the nickel doses were equivalent to 25 mg Ni/kg bw/day). No NOAEL could be determined. The study is considered to be of good quality and relevant for the evaluation of effects of nickel sulphate on the immune system.

4.4.1.3 Dogs

In a chronic dog study, groups of 3 male and 3 female beagle dogs were exposed to nickel sulphate hexahydrate by ingestion with diet (Ambrose et al. 1976). The dietary concentrations were 0, 100, 1000, and 2500 ppm Ni (equivalent to 0, 7.5, 75, or 188 mg Ni/kg bw/day (assuming that 1 ppm in diet equals 0.075 mg/kg bw/day). The dogs were exposed for 2 years.

At the 100 and 1000 ppm no effects were seen. The highest dosage level resulted in depressed body weight gain, this high dose caused vomiting and required stepwise increases from 1700 ppm to reach the final dose of 2500 ppm.
One high-dose male and female had polyuria when measured at the end of 2 years. Haematological values obtained at three-month intervals were variable but within normal range. There was a tendency towards lower haematocrit values in the highest dose group. Dogs in this group also showed high urine volume during the last two months on study.
Relative liver and kidney weights were statistically significantly increased (18% compared with control) in the highest dose group. In this group 5 of 6 dogs displayed multiple subpleural peripheral cholesterol granulomas, and 2 dogs had granulocytic hyperplasia of the bone marrow. No histopathological effects were found at the low and middle dose.
In this study, the **NOAEL** is 75 mg Ni/kg bw/day with a **LOAEL** of 188 mg Ni/kg bw/day (decreased body weight, lung granulomas, bone marrow hyperplasia). Although the study appears well performed, the small group size makes the interpretation of the pathological findings (e.g. lung granulomas) in relation to nickel sulphate difficult.

4.4.2 Nickel chloride

The following information has been extracted from the individual risk assessment report on nickel chloride (RAR chloride 2009). Table 6 gives an overview of the relevant repeated dose oral studies.

Groups of 30 male and 30 female Sprague-Dawley rats were given 0, 5, 35, or 100 mg Ni/kg bw/day as nickel chloride hexahydrate in water for 91 consecutive days by gavage (American Biogenics Corporation 1988). The study is described in a review, where it is stated that there is uncertainty whether the authors reported doses in terms of nickel or as nickel chloride, however argues that based on analytical determinations it appears that the doses were actually reported as nickel. All high-dose animals died at various times during study days 2-78; other toxicity data were not reported for these animals. Necropsy findings seen at a notably high incidence in this group included the following gastrointestinal tract abnormalities: green, dark, or dark green contents of the stomach, small intestine, caecum, colon, rectum, or entire gastrointestinal tract; stomach and/or small intestine distended and fluid filled; and stomach discoloured (red), ulcerated or eroded, and with smooth mucosa. At the mid dose, 6/30 males and 8/30 females died, deaths of 3/6 males and 5/8 were attributed to gavage error, based on histopathological analysis. Incidence of discoloured contents of the caecum and colon were also seen in treatment-related patterns in the low- and mid- groups. Lung abnormalities were seen in treatment-related patterns and generally were associated with animals dying on test. Other abnormalities seen at necropsy were generally few in number and not confined to any particular group. One male (day 44) and one female (day 92) died at the low dose. Salivation, lethargy, and irregular breathing were observed both in rats that died early and in those that survived.
Final body weight was significantly decreased in males and females at the mid-dose, to 81% and 92% of the corresponding controls, respectively. Food consumption was also decreased in males, but there was no statistically significant decrease in females. Significantly increased relative organ weights (adrenal, brain, testes in males; adrenals and heart in females) were apparently related to the decreased body weight. Similarly, decreased absolute organ weights (kidney, liver, and spleen in males; kidney in females) were probably related to the decreased body weight, in the absence of data indicating that nickel causes atrophy of these organs. Urine analysis evaluated protein, glucose, specific gravity, and pH; no

individual proteins in urine were evaluated. The study authors stated that a statistically and clinically significant increase in white blood cells was observed in males at the mid-dose at the interim sacrifice, and there was a minor increase in low and mid-dose females, but there was no effect at the terminal sacrifice. Blood glucose was statistically significantly decreased in mid-dose females at the terminal sacrifice, but not at the interim sacrifice or in males at any time point. Pneumonitis, characterized by intra-alveolar accumulation of pulmonary macrophages and degeneration of type II pneumocytes, was reported in mid-dose males (7/25) and females (10/25). However, histopathology was not conducted on low-dose animals. No histopathological effects in other organs were observed.
The **LOAEL** in this study was 5 mg Ni /kg bw/day (corresponding to 23 mg/kg bw/day as nickel chloride hexahydrate) based on death of one animal of each sex not attributed to gavage error, and the occurrence of signs of intoxication (salivation, lethargy, and irregular breathing).

Among a number of dose groups in a study designed to evaluate the promoting effect of metal compounds on rat renal tumorigenesis, a group of 15 male F344 rats received nickel chloride at a concentration of 600 ppm in the drinking water for 25 weeks (Kurokawa et al. 1985). Based on the water intake, the mean intake of elemental nickel was calculated as 10.2 mg/kg bw/day. There was no effect of nickel on survival, final body weight, or on absolute or relative kidney weight. Renal lesions suggestive of toxicity of nickel were not observed.
In this study examining a limited number of endpoints, 10.2 mg Ni/kg bw/day was a **NOAEL**.

4.5 Toxicity to reproduction

The information on reproductive toxicity of nickel sulphate and nickel chloride of relevance for the estimation of a health based quality criterion in drinking water for soluble inorganic nickel salts is summarised in Table 7 (fertility studies) and Table 8 (developmental toxicity studies) and further addressed in the text below the tables. No relevant studies regarding nickel nitrate have been found.

4.5.1 Nickel sulphate

The following information has been extracted from the individual risk assessment report on nickel sulphate (RAR sulphate 2009). Tables 7 and 8 give an overview of the relevant oral studies on fertility and developmental toxicity, respectively.

Following oral administration of 5.6 mg Ni/kg bw/day as nickel sulphate (25 mg/kg bw/day) to male rats for 4 months, the rats were caged with females in oestrus for 24 hours (Waltschewa et al. 1972). While 3/10 female rats caged with control males became pregnant, 0/10 females caged with nickel-treated males became pregnant. As a decrease in sperm count and testicular flaccidity were also reported, this study suggests a possible effect of nickel sulphate on male sex organs in rats. The **LOAEL** is 5.6 mg Ni/kg bw/day, the only dose level in the study.

An increase in abnormalities in spermatozoa from mice treated orally with a single dose of nickel sulphate (28 mg Ni/kg bw/day) was reported 5 weeks after treatment (Sobti & Gill 1989).

Table 7. Oral fertility studies with nickel sulphate and nickel chloride

Test type/ Exposure period	Route of exposure	Species	Doses	NOAEL parental	NOAEL fertility	Endpoint	Reference
Nickel sulphate							
3-generation	Diet	Rat	0, 250, 500, 1000 ppm Ni as nickel sulphate hexahydrate	500 ppm (40 mg Ni/kg bw/day)	1000 ppm (52-80 mg Ni/kg bw/day)	Fertility	Ambrose et al. (1976)
1-generation range finding	Gavage	Rat	0, 10, 20, 30, 50, 75 mg/kg bw/day as nickel sulphate hexahydrate	75 mg/kg bw/day (16.8 mg Ni/kg bw/day)	75 mg/kg bw/day (16.8 mg Ni/kg bw/day)	Fertility	SLI (2000a)
2-generation	Gavage	Rat	0, 1, 2.5, 5, 10 mg/kg bw/day as nickel sulphate hexahydrate	10 mg/kg bw/day (2.2 mg Ni/kg bw/day)	>10 mg/kg bw/day (2.2 mg Ni/kg bw/day)	Fertility and sperm quality	SLI (2000b)
Nickel chloride							
1-generation	Drinking water	Rat	0, 10, 50, 250 ppm as nickel chloride hexahydrate	-	250 ppm (31.6 mg Ni/kg bw/day)	Fertility	Smith et al. (1993)
2-generation	Drinking water	Rat	0, 50, 250, 500 ppm as nickel chloride hexahydrate	250 ppm (25 mg Ni/kg bw/day)	500 ppm (42 mg Ni/kg bw/day)	Fertility	RTI (1988)

Table 8. Oral developmental toxicity studies with nickel sulphate and nickel chloride

Test type	Route of exposure	Species	Doses	NOAEL maternal toxicity	NOAEL developmental toxicity	Reference
Nickel sulphate						
3-generation	diet	Rat	0, 250, 500, 1000 ppm Ni as nickel sulphate hexahydrate	500 ppm (40 mg Ni/kg bw/day)	< 250 ppm (13-20 mg Ni/kg bw/day) Neonatal mortality in F1	Ambrose *et al*. (1976)
1-generation range-finding	gavage	Rat	10, 20, 30, 50, 75 mg/kg bw/day as nickel sulphate hexahydrate	75 mg/kg bw/day (16.8 mg Ni/kg bw/day)	< 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) Neonatal mortality	SLI (2000a)
2-generation	gavage	Rat	1, 2.5, 5, 10 mg/kg bw/day as nickel sulphate hexahydrate	>10 mg/kg bw/day (2.2 mg Ni/kg bw/day)	5 mg/kg bw/day (1.1 mg Ni/kg bw/day) Peri-postnatal mortality in F1, but not F2 at 10 mg/kg bw/day (2.2 mg Ni/kg bw/day)	SLI (2000b)
Nickel chloride						
1-generation	Drinking water	Rat	0, 10, 50, 250 ppm as nickel chloride hexahydrate	-	LOAEL (equivocal): 10 ppm (1.3 mg Ni/kg bw/day) Pup mortality See text	Smith et al. (1993)
2-generation	Drinking water	Rat	0, 50, 250, 500 ppm as nickel chloride hexahydrate	250 ppm (25 mg Ni/kg bw/day)	No reliable NOAEL, see text	RTI (1988)

In a 3-generation reproduction study in Wistar rats, groups of 30 weanling rats per sex per group were fed 0, 250, 500, or 1000 ppm nickel as nickel sulphate hexahydrate for 11 weeks (Ambrose et al. 1976). Twenty females/group were mated individually with males from the same group for up to three successive 7-

day rotations. The F_{1a} pups were sacrificed and necropsied at weaning, and the parental (P_0) rats were re-mated to produce the F_{1b} generation. Mating of the F_{1b} and F_{2b} generations was as for the parental generation (17-20 rats mated/group). No food consumption data was reported and therefore only rough estimates of the animal's exposure can be made. In the NiPERA 2-generation study (see below), males consumed 25-28 g/day and females 18-20 g/day. Using an average body weight of 350 g and a food consumption of 18-28 g/animal/day, rough exposure levels of 0, 13-20, 26-40 and 52-80 mg Ni/kg bw/day can be calculated.
Body weights of the P_0 rats were slightly decreased at the high dose, with an average decrease of 13% reported for males and 8% reported for females. The fertility index was slightly lower at 250 and 1000 ppm in the F_{la} generation, and at 1000 ppm in the F_{2b} generation (around 60% compared to 70-79% in the controls), however, the differences were not statistically significant. The fertility index in exposed animals was similar to control values at the high dose in F_{1b}, F_{2a}, F_{3a} and F_{3b}.
The number of pups born dead was increased at all nickel doses in the F_{1a} generation and at 500 ppm and 1000 ppm in the F_{1b} generation, but there was no effect on pup mortality in later generations. There was a clear and consistent decrease averaging 27% in mean weanling body weight at 1000 ppm in all generations. The study authors state that there was no evidence of teratogenicity, based on gross examinations, and no histopathological effects on the F_{3b} generation, but present no supporting data.
Based on the results of the study, the **NOAEL** for effects on **fertility** seems to be 1000 ppm (52-80 mg Ni/kg bw/day), but due to the limited reporting of the data there is uncertainties concerning this NOAEL.
Evaluation of the developmental effects this study is complicated by the lack of statistical analyses and the reporting of results using pups rather than litters as the unit. Statistical analysis of the number of pups born dead show that the increased numbers at all doses levels in F_{1a} and at 500 and 1000 ppm in F_{1b} is statistically significant, see Table 9. Consequently, the **LOAEL** for **developmental** effects in this study is set to the lowest dose level investigated, i.e. 250 ppm (13-20 mg Ni/kg bw/day).

Table 9. Statistical analysis of the number of pups born dead in the 3-generation reproduction study by Ambrose et al. (1976)

	Dose	Litters	Born alive	Born dead	Born, total per litter	Born alive per litter	Born dead per litter	% Born dead per litter
F_{1a}	0	14	113	5	8.4	8.1	0.4	4.2%
	250	11	72	17*	8.1	6.5	1.5	19.1%
	500	14	96	13*	7.8	6.9	0.9	11.9%
	1000	12	93	16*	9.1	7.8	1.3	14.7%
F_{1b}	0	14	143	3	10.4	10.2	0.2	2.0%
	250	16	164	6	10.6	10.3	0.4	3.5%
	500	14	109	27*	9.7	7.8	1.9	19.9%
	1000	15	93	31*	8.3	6.2	2.1	25.0%

* p –values from 0.0-4.7%, Fishers exact test (rapporteur analysis)

A range-finding one-generation study in Sprague-Dawley rats was performed prior to the performance of the two-generation study described below (SLI 2000a). Groups of 8 males and 8 females were given nickel sulphate hexahydrate at doses of 0, 10, 20, 30, 50, 75 mg/kg bw/day by gavage. Dosing of P_0 animals began two

weeks prior to mating and dosing of F_1 offspring began on postnatal day 21. On lactation day 4, litters were randomly culled to a maximum of 8 pups.
No effects on P_0 survival, growth, gestation length, gross necropsy findings or fertility were observed.
Evaluation of post-implantation / peri-natal lethality among the offspring of treated parental rats (i.e. number of pups conceived minus the number of live pups at birth, see Table 10) showed statistically significant increases at the 30, 50, and 75 mg/kg bw/day dose levels. The values were also increased at the 10 and 20 mg/kg bw/day dose levels, however, the difference was not statistically significant. The mean live litter size was significantly decreased at 75 mg/kg bw/day. The number of dead offspring on lactation day 0 (stillbirth, see Table 10) was significantly increased in all exposure groups except the 50 mg/kg bw/day group.
The **NOAEL** for effects on **fertility** in this range-finding study is thus 75 mg/kg bw/day (16.8 mg Ni/kg bw/day).
The results of this range-finding study indicate a **LOAEL** for **developmental** effects (neonatal death) of 10 mg/kg bw/day (2.2 mg Ni/kg bw/day), the lowest dose level in the study.

Table 10. One-generation range-finding study (SLI 2000a)

Dose (mg/kg bw/day)	0	10	20	30	50	75
Post-implantation / peri-natal lethality[a]	0.4+0.3	2.6+1.9	1.6+0.6	2.3+0.8*	2.7+0.5**	4.8+0.8**
No. dead / live, day 0	1/128	12/100**	10/106**	10/92**	4/89	23/80**

a) mean + sem; * p< 5%; **p<1% (SLI 2000a)

The test substance was administered to F0 animals in a 2-generation reproduction study compliant with the OECD TG 416, Sprague-Dawley rats were administered nickel sulphate hexahydrate at dose levels of 1, 2.5, 5.0, and 10 mg/kg bw/day by gavage (SLI 2000b). The test substance was administered to the parental (P_0) generation before mating and during mating, pregnancy, and through the weaning of the first generation (F_1). At weaning, the administration was continued to F_1 offspring during growth into adulthood, mating and production of an F_2-generation, and up until the F_2-generation was weaned. On lactation day 4, litters were randomly culled to a maximum of 8 pups.
No effects on P_0 or F_1 growth and gestation length, fertility, sperm quality, oestrous cyclicity or sexual maturation were found, and there were no treatment-related clinical signs of toxicity or histopathological changes in liver, reproductive organs, or other tissues examined in the study.
The post-implantation / peri-natal lethality until postnatal day 0 among the F_1 offspring (i.e. number of pups conceived minus the number of live pups at birth, see Table 11) was higher at 10 mg/kg bw/day, however, the difference was not statistically significant (2.1 at 10 mg/kg bw/day vs. 0.9 in the control group, p = 8.6% in Mann-Whitney test). In F2 offspring, the value for post-implantation / peri-natal lethality was similar to the F2 control value.

This 2-generation study is well described and well performed. However, since the highest dose level did not induce any signs of toxicity in the P_0 animals, the study does not fulfil OECD TG concerning the dose levels used. Therefore, the results of the study are not conclusive concerning the potential for effects of nickel sulphate on **fertility** at higher dose levels than the **NOAEL** of 10 mg/kg bw/day (2.2 mg Ni/kg bw/day).

Table 11. Two-generation study, F_1 offspring (SLI 2000b)

Dose	0	1	2.5	5	10
Postimplantation/ perinatal lethality[a], day 0	0.9+0.2	1.5+0.4	1.2+0.3	1.3+0.2	2.1+0.4
Postimplantation/ perinatal lethality[a], day 4 (%)	1.0+0.2 (7.1+1.5%)	1.2+0.2 (8.1+1.4%)	1.2+0.3 (8.7+2.0%)	1.4+0.2 (11.0+2.2%)	2.3+0.4** (15.8+2.8%)*

a) mean + sem; * p< 5% (Rapporteur statistics); **p<1% (Sommer et al. 2002), see also Appendix 2.

The authors state that the results indicate that the highest dose of 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) was a NOAEL for the developmental end points studied, including the variable of post-implantation / peri-natal lethality. As peri-natal lethality also occurs after the day of birth, the Danish EPA wanted to evaluate the whole time period from implantation to peri-natal day 4 as a continuum to which NiPERA agreed. For the dose group of 2.2 mg Ni/kg bw/day the post-implantation / peri-natal lethality is 2.29+0.43 (mean + sem) per litter and for the control group it is 1.00+0.22 per litter. The statistical analysis gives a p-value of 5.8% in Mann-Whitney test***. See Appendix 2 for further statistical analyses of the developmental end points.***

In conclusion, statistical analysis using the litter as the unit of significance shows that there is a statistically significant increase in litters with high post-implantation / peri-natal lethality and in the mean percentage post-implantation / peri-natal lethality in F_1 group dosed with 2.2 mg Ni/kg bw/day.
There was no statistically significant effect on post-implantation / peri-natal lethality in F_2 offspring. However, the parental animals for this generation were selected from the F_1 generation and obviously the F_1 offspring that died pre- or post-natally are not represented. Consequently, the animals that may have had the highest sensitivity to the effect may not have been included in the production of F_2.
Based on the supplementary statistics using the litter as the statistical unit and showing that the increase in post-implantation / peri-natal lethality in F_1 is statistically significant as well as the above consideration concerning the finding of effects in F_1 but not in F_2, it is evaluated that the 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) cannot be regarded as a clear NOAEL. Consequently, the **NOAEL** is set to 5 mg/kg bw/day (1.1 mg Ni/kg bw/day) in this study.
Since the highest dose level in this 2-generation study did not induce any signs of toxicity in the P_0 animals, the study does not fulfil the OECD TG 416 concerning the dose levels used. As the results of the prior range-finding one-generation study indicate that post-implantation / peri-natal lethality is increased in the absence of maternal toxicity, it is considered acceptable for the evaluation of developmental toxicity that the highest dose level did not induce maternal toxicity.

4.5.2 Nickel chloride

The following information has been extracted from the individual risk assessment report on nickel chloride (RAR chloride 2009). Tables 7 and 8 give an overview of the relevant oral studies on fertility and developmental toxicity, respectively.

An increase in abnormalities was reported in spermatozoa from mice treated orally with a single dose of nickel chloride (43 mg Ni/kg) (Sobti & Gill 1989). Spermatozoa were examined 5 weeks after treatment. Because mg/kg doses were given, it is assumed that treatment was by gavage. Due to the use of a single dose only, the effects level of 43 mg/kg cannot be considered as a **LOAEL**.

Pandey & Srivastava (2000) administered 0, 5, 10 or 20 mg/kg nickel chloride 5d/w for 35 days to groups of 6 young male mice. Dose related effects on sperm motility and count as well as decreased body weight gain were observed at 10 and 20 mg/kg bw/day. There also seemed to be an increase in abnormal sperm at the same dose levels. No effects were observed at 5 mg/kg bw/day. This study indicates a **NOAEL** of 5 mg/kg bw/day for effects on sperm, however, due to the low number of animals per group this cannot be considered as a reliable NOAEL.

In a 1-generation reproductive toxicity study, female Long Evans rats were administered 0, 10, 50, or 250 ppm nickel as nickel chloride hexahydrate in drinking water (Smith et al. 1993). Groups of 34 females were administered the nickel for 11 weeks prior to breeding, mated with experienced, unexposed males. Exposure of the females continued during two successive gestation periods (G1 and G2) and lactation periods (L1 and L2). The overall average doses were reported as 0, 1.3, 6.8, and 31.6 mg Ni/kg bw/day.
Average water intake was unaffected except at the high dose. Average doses were about 20% lower during pre-breeding and gestation, and about 60% higher during lactation, due to higher water consumption during lactation. Maternal body weight on GD 21 was statistically significantly reduced by around 6% at the high dose in G1, but not in G2. Statistically significant decreases in body weight gain during gestation were observed at the mid and high doses during G1 (36.9% at 50 ppm, 37.7% at 500 ppm versus 41.8% in control group), but similar weight gains were found in all groups during G2. A small, but statistically significant decrease in prolactin was observed in high-dose dams. There was no treatment-related effect on reproductive performance indices (mating success, rate of impregnation).
There was no treatment-related effect on mean pup birth weight or weight gain in either generation. However, there was a dose-related increase in both the number and proportion per litter of pups either born dead or dying shortly thereafter (trend analysis: G1, $P < 0.001$, 0.04; G2, $P < 0.03$, 0.02), see Table 12. The total number of dead pups and the proportion of dead pups per litter were significantly increased at the high dose in both the first and second breeding. There was no effect on other measures of pup mortality in the first generation, but the total number of dead pups and the percentage of dead pups per litter on postnatal day 1 were statistically significantly increased at all doses in the second generation, and the number of litters with dead pups was also borderline significant ($p<0.06$) at the low dose of the second generation.
The **NOAEL** for **fertility** in this study was 31.6 mg Ni/kg bw/day, the highest dose level in the study. Effects on sperm quality and oestrus cyclicity were not investigated in this study.
The inconsistency between generations makes it difficult to identify a clear NOAEL or LOAEL for **developmental** toxicity in this study. However, as all three measures of pup death were statistically significant or borderline significant at the low dose in the second generation an equivocal **LOAEL** for this study was 1.3 mg Ni/kg bw/day.

Nickel chloride hexahydrate was administered to male and female CD rats (30/sex/dose) at 0, 50, 250, or 500 ppm nickel in drinking water in a 2-generation study (RTI 1988). An additional dose level of 1000 ppm was eliminated after 2 weeks due to excessive toxicity. The parental animals were exposed from 11 weeks before cohabitation, and exposure continued for a total of 24 weeks (males) or 30 weeks (females). Groups of 10 rats/sex comprised a satellite sub-chronic non breeder study. The average nickel consumption reported by the authors varied by more than a factor of 2, with the highest consumption at the beginning of the pre-mating exposure and during the latter part of the lactation period. As a conservative estimate, the average exposure during gestation, which was on the

low end of overall exposure levels, was used as the dose level for each group. This choice also takes into account the possibility that gestational exposure alone could have accounted for observed effects. Thus, the estimated doses were 0, 6.0, 25, and 42 mg Ni/kg bw/day.

Table 12. Pup mortality data and p-values in the Smith et al. (1993) study

Dose (ppm Ni)	No. of litters with dead pups at birth (no. of viable litters)	Total dead pups PD 1 (% dead pups per litter)	Total dead pups PD 21 (% dead per litter)
G1			
0	5 (25)	5 (1.7%)	38 (11.5%)
10	5 (25)	9 (3.1%)	32 (7.6%)
50	0 (24)	0 (0.0%)	10 (2.8%)
250	11^{+} (27)	35*** (13.2%)**	55 (15.0%)
G2			
0	2 (23)	2 (1.0%)	22 (12.5%)
10	7^{+} (22)	11** (4.3%)**	33 (13.4%)
50	6 (24)	16* (4.6%)$^{+}$	61 (19.4%)
250	10** (25)	22*** (8.8%)***	69 (29.9%)**

$^{+}$ 0.05 <P < 0.10; * 0.03 < P < 0.05: ** 0.01 < P < 0.03; *** 0.001 <P < 0.01

At the 500 ppm dose level there was a statistically significant decrease in the P_0 body weight in males and females (95% and 90% of controls, respectively), along with decreased absolute and relative liver weights in the females (90% and 89% of controls, respectively), but not in the males. There was no treatment-related effect on reproductive performance indices (mating success, rate of impregnation), reproductive organ weights or histopathology of reproductive organs.
In the F_{1a} generation at the 500 ppm dose level, the number of live pups/litter was significantly decreased, pup mortality was significantly increased, and average pup body weight was significantly decreased in comparison with controls, see Table 13. Although there was no statistically significant effect at 250 ppm, there was some indication of decreased number of live pups/litter. Similar effects were seen with F_{1b} litters of P_0 dams exposed to 500 ppm nickel. In the 50 and 250 ppm dose groups, increased pup mortality and decreased live litter size was observed in the F_{1b} litters. However, these effects seen in the F_{1b} litters are somewhat questionable because the room temperature was 3-5°C higher than normal at certain times (gestation-postnatal days) along with lower levels of humidity. Therefore, the above results seen at 50 and 250 ppm may not be adverse effects of nickel only.
F_{1b} males and females were randomly mated (19-30/sex/group) on postnatal day 70 and their offspring (F_{2a} and F_{2b}) were evaluated through postnatal day 21 or on gestational day 21. This phase included teratological evaluations of F_{2b} foetuses. The average gestational nickel consumption of F_{1b} dams was 0, 6.2, 23, and 42 mg Ni/kg. Evaluation of the data indicated that the 500 ppm dose caused significant body weight depression of both mothers and pups and increased neonatal mortality during the postnatal development period. No effects on prenatal growth or viability were observed in F_{2b}. The percent foetuses malformed per litter were significantly increased at 50 ppm, due primarily to a higher incidence of short rib in that group. In the absence of similar effects at higher doses, the increased incidence at 50 ppm is probably not due to exposure to nickel.
The **NOAEL** for the **parental** generation was 250 ppm (25 mg Ni/kg bw/day) and the **NOAEL** for **fertility** was 500 ppm (42 mg Ni/kg bw/day). Effects on sperm quality and oestrus cyclicity were not investigated in this study.

Regarding developmental toxicity, the study shows that exposure to nickel can cause increased neonatal mortality at 42 mg Ni/kg bw/day and possibly at lower doses of 6 and 25 mg Ni/kg bw/day, but a reliable **NOAEL** for **developmental** toxicity cannot be identified in this study.

Table 13. Litter size and pup mortality in RTI (1988) study

Dose (ppm Ni)	Live pups per litter, PND 1 (No. of litters)	Live pups per litter, PND 4	% mortality per litter, PND 1-4
F_{1a} litters			
0	13.3 (26)	13.0	2.1%
50	14.0 (25)	13.8	1.2%
250	11.5 (23)	11.3	1.7%
500	10.9* (27)	8.8**	18.4%**
F_{1b} litters			
0	15.3 (15)	15.1	0.9%
50	11.8* (19)	11.4*	7.2%
250	11.5* (19)	11.3*	8.6%
500	9.5** (15)	5.3**	53.3%**
F_{2a} litters			
0	13.6 (24)	12.3	12.9%
50	14.2 (28)	13.5	4.4%
250	12.4 (25)	11.7	9.8%
500	11.4* (15)	8.3*	28.6%

* P < 0.05; ** P < 0.01

4.6 Mutagenic and genotoxic effects

The mutagenicity and genotoxicity of nickel compounds have been extensively studied. Below, the available data regarding the three prioritised soluble inorganic nickel salts (nickel sulphate, nickel chloride, and nickel nitrate) are summarised.

4.6.1 *In vitro* studies

4.6.1.1 DNA damage and repair

The *in vitro* data on DNA damage and repair are summarised in Table 14.

Most of the data comes from studies with nickel chloride. There are some studies in bacteria showing differential toxicity between repair deficient and normal strains. There are positive studies of gene conversion in yeast, and a series of studies showing induction of DNA single strand breaks in mammalian cells. There is also evidence of DNA synthesis inhibition, disturbance of DNA damage recognition and inhibition of DNA repair. Human cells appear to be more resistant to nickel induced strand breakage than hamster cells.

There is evidence indicating that nickel ions bind to DNA. The nickel ion also binds to chromatin more strongly than to naked DNA. There is evidence of nickel catalysed oxidative damage to DNA.

Table 14. Summary of results of *in vitro* tests for DNA damage and repair

	Nickel sulphate	Nickel chloride	Nickel nitrate
Prokaryotes			
E. coli, differential toxicity in repair deficient strain		positive	negative
Fungi			
Yeast, gene conversion	positive	positive	
Mammalian cells			
Rat liver epithelial cells (inhibition of DNA synthesis)		positive	
Hamster CHO cells (DNA damage)		positive	
SHE (Strand breaks)		positive	
Human bronchial epithelial cells (inhibition of DNA synthesis)	positive		
Human diploid fibroblasts (DNA damage)	negative	negative	
Human gastric mucosal cells (DNA damage)	negative		
Human peripheral lymphocytes (DNA strand breaks)		equivocal	
Human lung cells (inhibition of DNA repair)		positive	

4.6.1.2 Gene mutation

The *in vitro* data on gene mutation are summarised in Table 15.

Nickel chloride has been tested extensively in bacteria, and most of the available data comes from studies with this nickel compound. In general, the nickel compounds tested gave negative results in bacterial assays with *S. typhimurium* and *E. coli.* The overall evidence indicates that nickel compounds are not mutagenic in bacteria.

Both nickel sulphate and nickel chloride have been tested in gene mutation studies with mammalian cell lines. Many of these studies showed positive results, although these were often weakly positive. In some cases, only certain loci were affected (e.g. a positive result at the tk_{slow} locus, but not at the tk_{normal} or hprt loci). Whilst these results may indicate gene mutation, the positive results in at least some of these assays could possibly be due to other genetic events (chromosomal aberrations and DNA methylation) than point mutations. For instance, it has been shown that the increases in mutant frequency seen at the *gpt* gene of v79 cells were due to changes in DNA methylation. DNA methylation seems to be related to the inhibition of tumour suppression genes.

4.6.1.3 Chromosomal effects

The *in vitro* data on chromosomal effects are summarised in Table 16.

This effect has been extensively studied with both nickel chloride and nickel sulphate. There are slightly more studies of chromosomal aberrations (CA) with nickel chloride than with nickel sulphate; the reverse is true for studies of sister chromatid exchange (SCE).

Positive results were seen in almost all studies of CA and SCE. Effects have also been seen on spindle function, suggesting that numerical changes might occur.

4.6.1.4 Cell transformation

The *in vitro* data on cell transformation are summarised in Table 17.

Most of the data on cell transformation comes from studies on nickel sulphate, although there are additional studies with nickel chloride. Many of these studies indicate an effect on cell transformation, anchorage independence and loss of cell communication.

Table 15. Summary of results of *in vitro* tests for gene mutations

	Nickel sulphate	Nickel chloride	Nickel nitrate
Prokaryotes			
S. typhimurium	negative	negative (4)	negative
E. coli	negative	negative	
Corynebacterium		positive	
Host mediated assay			
Salmonella in NMRI mice		negative	
Serratia marcescens in NMRI mice		negative	
Fungi			
S. cerevisiae	negative	positive	
Mammalian cells			
CHO cells		equivocal	
Mouse lymphoma cells (TK+/-)	positive (1)	positive	
SHE cells	negative (2)		
V79 cells	positive (1)	positive	
Rat NRK cells		positive	
Rat 6m2 murine sarcoma virus infected cells		positive	
Rat liver epithelial cells		positive	
Human lymphoblasts	positive (3)		

1) weakly positive
2) co-mutagenic with benzo(a)pyrene
3) for the tk_{slow} locus only
4) a fluctuation test gave a positive result

Table 16. Summary of results of *in vitro* tests for chromosomal effects

	Nickel sulphate	Nickel chloride	Nickel nitrate
Chromosomal aberrations			
Pisum			positive
Mouse mammary carcinoma cells (FM3A cells)		positive	
Rat lung epithelial cells	positive		
CHO cells		positive	
SHE cells	positive		
Human lymphocytes	positive		
Human bronchial epithelial cells		positive	
Sister chromatid exchanges			
Mouse mammary carcinoma cells (FM3A cells)	positive	positive	
CHO cells	positive	positive	
SHE cells	positive		
Human lymphocytes	positive	positive	
Spindle disturbance			
Rat embryo cells		positive	
human peripheral lymphocytes	positive		
Micronucleus test (kinetochore stained)			
Human diploid fibroblasts	weak positive	weak positive	

Table 17. Summary of results of *in vitro* tests for cell transformation and other effects

	Nickel sulphate	Nickel chloride	Nickel nitrate
BHK 21 cells			
SHE cells	positive	positive	
mouse embryonic fibroblasts	negative	negative	
Rat embryo cells	positive		
Hamster V79, loss of cell communication	positive	positive	
Human foreskin cells (anchorage independence)	positive		

4.6.2 *In vivo* studies

4.6.2.1 DNA damage and repair

The *in vivo* data on DNA damage and repair are summarised in Table 18.

There is evidence that soluble nickel compounds can give rise to both DNA breaks and DNA-protein crosslinks *in vivo*. Recent studies have shown that nickel chloride induced single and double stranded DNA breaks as measured by the Comet assay in leucocytes in mice after oral administration.

Table 18. Summary of results of *in vivo* tests for DNA damage and repair

	Nickel sulphate	Nickel chloride	Nickel nitrate
Rat, mouse, DNA strand breakage	positive	positive	
Mouse, inhibition of DNA synthesis:			
kidney epithelium	negative		
liver epithelium	positive		
Hamster, suppression of DNA synthesis		positive	

4.6.2.2 Gene mutation

The *in vivo* data on gene mutation are summarised in Table 19.

The only *in vivo* studies for gene mutations with soluble nickel compounds have been carried out in *Drosophila melanogaster*. Weakly positive effects have been seen in one study. This is consistent with the data seen *in vitro*.

Table 19. Summary of results of *in vivo* tests for gene mutation

	Nickel sulphate	Nickel chloride	Nickel nitrate
Drosophila			
Somatic eye colour test		negative	negative
Wing spot mutation		positive(1)	
Mutation		negative	equivocal

1) weakly positive

4.6.2.3 Chromosomal effects

The *in vivo* data on chromosomal effects are summarised in Table 20.

Data for the evaluation of chromosomal effects *in vivo* is mainly available for nickel sulphate, chloride and nitrate.
Chromosomal aberrations have been seen *in vivo* in a number of studies. This effect has been seen with the three soluble substances tested. There is also evidence, sometimes with mixed exposure, to show that this effect is also seen in humans.

The data from micronucleus tests is conflicting. One of the studies, which was carried out as part of an international collaborative study, found a negative result for both nickel sulphate and nickel chloride. A micronucleus study of nickel sulphate in rats after oral administration using the Annex V B12 (OECD 474) protocol was also negative. A third study also found no effect on micronucleus induction. A number of largely Indian studies have all shown positive results.

The data from dominant lethal tests suggests that there is no significant dominant lethal effect, although the soluble nickel compounds tested may reduce fertilisation rate after intraperitoneal administration. One study showed a significant increase in sperm head anomalies.

There is therefore *in vivo* data confirming the clastogenicity seen *in vitro*.

Table 20. Summary of results of *in vivo* tests for chromosomal effects

	Nickel sulphate	Nickel chloride	Nickel nitrate
Plants			
Vicia faba (mitotic effects)	positive		
Insects			
Drosophila	positive		
Mammals – Chromosomal aberrations			
Mouse	positive	positive	Positive
Rat	negative		
Hamster		positive	
Human	positive	positive	
Mammals – Sister chromatid exchanges			
Mouse			
Rat			
Human	negative		
Mammals – Micronucleus			
Mouse	conflicting data	conflicting data	conflicting data
Rat	negative		
Mammals – Dominant lethal test			
Mouse		negative	Negative
Rat		negative	

4.7 Carcinogenic effects

4.7.1 Oral administration

The carcinogenicity of **nickel sulphate** following oral administration has been studied in rats and dogs; these studies are summarised in Table 21. An oral

(gavage) OECD TG 451 carcinogenicity study in rats did not show any treatment related increase in tumours related to the exposure. Furthermore, no neoplasms were revealed in either rats or dogs in two older non-guideline dietary studies; however, these studies are limited because of the low number of animals (rats and dogs), the high mortality in all groups of rats (causes of death not reported) resulting in only a small number of animals being exposed for the total period of 2 years and being available for sacrifice and histopathology, and the limited reporting of the study design and results.

Table 21. Summary of oral carcinogenicity studies of nickel sulphate hexahydrate in experimental animals

Route of administration	Species, group size and sex	Concentration, exposure duration	Results	Reference
Oral, dietary	Wistar rats 25 males and females per group	0, 100, 1000 or 2500 ppm Ni in feed 2 years	No treatment related neoplasms observed	Ambrose et al. (1976)
Oral, dietary	Beagle dogs 3 males and females per group	0, 100, 1000 or 2500 ppm Ni in feed 2 years	No treatment related neoplasms observed	Ambrose et al. (1976)
Oral, gavage	Fischer rats 60 males and females per group	0, 2.2, 6.7, 11 mg Ni/kg bw/day 2 years	No treatment related neoplasms observed	CRL (2005)

No data regarding carcinogenicity of **nickel chloride** and **nickel nitrate** following oral administration in experimental animals have been located.

4.7.2 Inhalation

There is no evidence of carcinogenic activity following inhalation of **nickel sulphate** hexahydrate in rats and mice under the conditions of the available inhalation studies.

The studies in experimental animals on the carcinogenicity of **nickel metal** following inhalation or intratracheal instillation suffer from inadequacies and are considered inadequate for an evaluation of the carcinogenic activity of nickel metal following inhalation.

No studies regarding carcinogenic activity following inhalation exposure or intratracheal instillation of **nickel chloride**, **nickel nitrate**, and **nickel carbonate** in experimental animals have been located.

In conclusion, the available data on carcinogenicity of various nickel compounds is considered as being insufficient for a conclusion on the carcinogenic potential of the five prioritised nickel compounds (nickel sulphate, nickel chloride, nickel nitrate, nickel carbonate, and nickel metal) in experimental animals following inhalation.

Inhalation studies on **nickel oxide** and **nickel subsulphide** showed some evidence and clear evidence, respectively, for carcinogenic activity following inhalation in rats, and there was equivocal evidence for nickel oxide in female mice.
It should be noted that soluble forms of nickel might differ from insoluble forms of nickel in carcinogenic potential or in potency in experimental animals following

exposure by inhalation; however, the available data are not sufficient for an evaluation of this suggestion.

4.7.3 Initiator-promoter studies

Three studies evaluating the promoting effect of **nickel sulphate** in experimental animals have been located, which may indicate a promoter effect of nickel sulphate, if applied locally to the nasopharynx or the oral cavity, or by the feed to pups from initiated dams; however, the indications are rather weak.

In a two-stage carcinogenesis assay, orally administered **nickel chloride** enhanced the renal carcinogenicity of N-ethyl-N-hydroxyethylnitrosamine in rats, but not the hepatocarcinogenicity in rats after initiation with N-nitrosodiethylamine, the gastric carcinogenicity in rats after initiation with of N-methyl-N-nitro-N-nitrosoguanidine, or the pancreatic carcinogenicity in Syrian golden hamsters following initiation with N-nitrosobis(2-oxopropyl)amine.

5 Regulations

5.1 Ambient air

Denmark (C-value): 0.0001 mg/m^3 (nickel measure as Ni) (MST 2002).

WHO (2000): Nickel compounds are human carcinogens by inhalation exposure. The present data are derived from studies in occupationally exposed human populations. Assuming a linear dose-response, no safe level for nickel compounds can be recommended. On the basis of the most recent information of exposure and risk estimated in industrial populations, an incremental risk of 3.8 x 10^{-4} can be given for a concentration of nickel in air of 1 µg/m^3. The concentrations corresponding to an excess lifetime risk of 1:10000, 1:100000, and 1:1000000 are about 250, 25, and 2.5 ng/m^3, respectively.

5.2 Drinking water

Denmark: 20 µg Ni/l (drinking water) (MM 2007)

WHO (2008): 0.07 mg/l
"Based on a NOAEL of 1.1 mg Ni/kg bw/day for all end-points studied, including the variable of post-implantation/perinatal lethality (SLI 2000, EU 2004). By application of an uncertainty factor of 100 (10 to account for interspecies variation and 10 to account for intraspecies variation) gives a TDI of 11 µg/kg bw/day. Data show that the exposure from food is moderate and that a higher exposure could be allowed from drinking-water in consideration of the circumstances when naturally elevated nickel is present. A health-based value of 70 µg/litre (rounded from 66 µg/litre) could be determined from this TDI by assuming a 60-kg adult drinking 2 litres of water per day and allocating 20% of the TDI to drinking-water.
The LOAEL established after provocation of fasted patients with an empty stomach is 12 µg/kg bw (Nielsen et al. 1999). Because this is based on a highly sensitive population, it is not necessary to include an additional uncertainty factor. Based on these data, the guideline value, to allow for nickel-sensitive individuals, can be calculated as 70 µg/litre (rounded valued), assuming a 60-kg adult drinking 2 litres of water per day and allocating 20% of total daily intake to drinking-water. Although this is very close to the acute LOAEL, established by Nielsen et al. (1999), the absorption from drinking-water is 10- to 40-fold higher than the absorption from food, and basing the total acceptable

intake for oral challenge from studies using drinking water on an empty stomach in fasted patients can be considered a worst-case scenario.
Based on consideration of both the animal and human data, assuming a 60-kg adult drinking 2 litres of water per day and making an allocation of 20% of the TDI to drinking-water in order to allow for circumstances where naturally elevated nickel occurs in drinking-water, the guideline value is 70 µg/litre. It must be emphasized that it is not appropriate to allow nickel concentrations to be increased to the guideline value from pollution or where cost-effective controls are available."

5.3 Soil

Denmark: 30 mg/kg (nickel unspecified) (MST 2008)

5.4 Occupational Exposure Limits

Denmark: 0.01 mg Ni/m^3 notation K (carcinogenic) (nickel compounds, soluble) (revised 2000) (At 2007)

5.5 Classification

Nickel sulphate:
Carc. Cat. 1;R49, Muta. Cat. 3;R68, Repr. Cat. 2;R61, T;R48/23, Xn;R20/22, Xi;R38, R42/43, N;R50-53.

Nickel chloride:
Carc. Cat. 1;R49, Muta. Cat. 3;R68, Repr. Cat. 2;R61, T;R23/25-R48/23, Xi;R38, R42/43, N;R50-53.

Nickel nitrate:
O;R8, Carc. Cat. 1;R49, Muta. Cat. 3;R68, Repr. Cat. 2;R61, T;R48/23, Xi;R38-41, R42/43, N;R50-53.

5.6 IARC

Nickel compounds are carcinogenic to humans (Group 1). There is sufficient evidence in humans for the carcinogenicity of nickel sulphate, and of the combinations of nickel sulphides and oxides encountered in the nickel refining industry. There is sufficient evidence in experimental animals for the carcinogenicity of metallic nickel, nickel monoxides, nickel hydroxides and crystalline nickel sulphides. There is limited evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selenides and nickel telluride. The Working Group made the overall evaluation on nickel compounds as a group on the basis of the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and several types of other relevant data supported by the underlying concept that nickel compounds can generate nickel ions at critical sites in their target cells. (IARC 1990).

The IARC Working Group has recently (2009) reaffirmed the classification of nickel compounds “as carcinogenic to humans” (Group 1) due to tumours in lung, nasal cavity and paranasal sinuses. It is mentioned that studies involved complex occupational exposures to a metal and its compounds, making it impossible to separately assess their carcinogenicity. (Straif et al. 2009).

5.7 US-EPA

Oral reference dose (RfD): 0.02 mg Ni/kg bw/day (last revised 1996).
The RfD is based on a NOAEL of 100 ppm nickel in the diet, corresponding to 5 mg Ni/kg bw/day (Ambrose et al. 1976) by using a conversion factor that 1 ppm = 0.05 mg/kg bw/day assuming rat consumption. An uncertainty factor of 10 is used for interspecies extrapolation and 10 to protect sensitive populations. An additional uncertainty factor of 3 is used to account for inadequacies in the reproductive studies. The nickel dietary study by Ambrose et al. (1976) identifying a NOAEL of 100 ppm (5 mg/kg bw/day) is supported by the sub-chronic gavage study in water (American Biogenics Corp. 1986), which indicated the same NOAEL (5 mg/kg bw/day). (IRIS 2008).

5.8 WHO / JECFA

No monograph(s) on nickel compounds were located.

6 Summary and evaluation

6.1 Description

Nickel can be found in a variety of oxidation states ranging from 0 to IV. However, Ni (II) is the only oxidation state occurring in ordinary chemistry. Ni (II) forms a wide variety of compounds ranging from simple inorganic complexes (salts) to complexes with various organic ligands. In the aqueous chemistry Ni (II) is the only oxidation state that has to be considered. In the absence of strong complexing agents Ni (II) appears in aqueous solution as the green hexaquonickel (II) ion Ni $(H_2O)_6^{2+}$.
In this evaluation only soluble inorganic nickel salts are considered in relation to an estimation of a health based quality criterion in drinking water.

6.2 Environment

Nickel and its compounds are naturally present in the earth's crust. Nickel is released to the environment from natural processes as well as from anthropogenic activities.

In Denmark, nickel was detected in 3362 of 6972 wells analysed for nickel in a period from 1993-2002. In 221 wells, the drinking water limit at 20 µg Ni/l was exceeded. The median value was 2 µg Ni/l and the 95th percentile was 8.8 µg Ni/l. The highest detected value was 590 µg Ni/l.

Based on two European reference sources (the EMEP database and the Airbase database), the typical nickel ambient air concentrations derived was 4.5 ng Ni/m^3.

The 50th percentile value for measured soil concentrations in Europe was 14 mg Ni/kg soil (dry matter).
Based on the results of studies on nickel concentrations in house dust and street dust, typical concentrations of 49 mg Ni/kg and of 115 mg Ni/kg was derived for house dust and street dust, respectively.

Nickel concentrations in foods can vary widely, depending on the food type, conditions and location under which the food products were produced and stored. Rich food sources of nickel are oats, chocolate, breakfast cereals, bread, nuts, dried beans, peas and pods, other grains and some vegetables. Products of animal origin generally contain low nickel concentrations.

6.3 Human exposure

Human exposure to nickel can result from inhalation, consumption of food and drinking water, or incidental ingestion of soil or dust contaminated with nickel. Food is the predominant source of exposure to nickel for both adults and young children.

6.4 Toxicokinetics

The extent of absorption from the gastrointestinal tract is influenced by whether the nickel compound is administered in drinking water, to fasting subjects, or together with food. Therefore, different conclusions on the absorbed fraction of nickel for fasting and non-fasting individuals are drawn.

Absorption of nickel following oral ingestion of nickel sulphate has been evaluated in a number of human studies; no human data are available for the other prioritised soluble compounds (nickel chloride and nickel nitrate). One study has shown that absorption of nickel can be as high as 27% when nickel sulphate was administered in drinking water to fasting individuals compared with about 1%, when administered together with food to fasting individuals. Another human study on volunteers, in which the nickel compound administered was not specified, showed similar results with 25.8% of the administered dose being excreted in the urine following administration of nickel in drinking water to fasting individuals compared with 2.5% when nickel was mixed into a meal.
Based on experimental data from various human studies, a biokinetic model has been used to estimate nickel absorption; the results showed that estimated nickel absorption ranged from 12-27% of the dose when nickel was ingested after a fast, to 1-6% when nickel was administered either in food, in water, or in a capsule during (or in close proximity to) a meal.
One study in rats showed an absorption of 11% for nickel sulphate, of 9.8% for nickel chloride, and of 34% for nickel nitrate when the compound was administered in a 5% starch saline solution. Other studies of nickel chloride in experimental animals have shown an absorption of 3-6% in rats (non-fasting, gavage in 0.1N hydrochloric acid) and of 1.7-10% in mice (gavage).

No information exists on the human tissue distribution of nickel after oral exposure. Animal studies show that after the ingestion of soluble nickel salts, the highest nickel level is observed in the kidney.

Following oral exposure, the elimination of nickel is primarily in the faeces due to the relatively low gastrointestinal absorption. Urinary excretion is usually the major clearance route for absorbed nickel. Other routes of elimination, which are of minor importance, include hair, saliva, sweat, tears, and milk.

6.5 Human toxicity

6.5.1 Single dose toxicity

In the 19th century, nickel salts were used medicinally, and 325 mg nickel sulphate in solution or in pill form was reported to produce nausea and giddiness.

6.5.2 Irritation

Human data indicate that nickel sulphate and nickel chloride can induce skin irritation. No human data have been found regarding irritation to eyes and the respiratory tract.

6.5.3 Sensitisation

Nickel is well known as a skin sensitiser, and is one of the most frequent skin sensitisers in man. The nickel ion is considered exclusively responsible for the immunological effects of nickel.
Systemic exposure to nickel orally in individuals without contact allergy to nickel does not result in sensitisation but may result in immunological tolerance, meaning that the individual is unable to develop contact allergy to nickel at subsequent exposures.
When nickel was given orally to nickel allergic patients with hand eczema, worsening of their hand eczema was observed.

Five single cases of work related asthma due to exposure to nickel sulphate in electro- or metal plating have been reported.

6.5.4 Repeated dose toxicity

There are no relevant human data.

6.5.5 Toxicity to reproduction

There are no relevant human data.

6.5.6 Mutagenic and genotoxic effects

No human *in vivo* studies have been located. Tests with human cells *in vitro* are summarised in section 6.6.6.

6.5.7 Carcinogenic effects

No human data regarding carcinogenicity of nickel compounds following oral ingestion have been located.

6.6 Animal toxicity

6.6.1 Single dose toxicity

The oral LD_{50} values for the three soluble nickel salts, nickel sulphate, nickel chloride and nickel nitrate, tested as hydrates in the rat, ranged from 175 to 1620 mg compound/kg. The corresponding figures expressed as mg Ni/kg were 43 –330.
A toxic class method study showed no mortality or signs of toxic symptoms at 200 mg nickel nitrate hexahydrate/kg.

6.6.2 Irritation

Nickel sulphate and nickel nitrate are skin irritants, whereas nickel metal did not cause skin irritation. Nickel nitrate is a severe eye irritant. Nickel sulphate caused lung inflammation and degeneration of the olfactory epithelium in rats and mice following relatively short periods of exposure (16 days).

6.6.3 Sensitisation

Studies on skin sensitisation have shown that nickel sulphate is a skin sensitiser in guinea pigs and mice and that nickel chloride is a skin sensitiser in guinea pigs.

No data regarding respiratory sensitisation in animals have been located.

6.6.4 Repeated dose toxicity

Repeated dose toxicity studies with oral exposure to nickel sulphate have been conducted in rats, mice and dogs and to nickel chloride in rats. Table 6 gives an overview of the relevant repeated dose toxicity oral studies.

For **nickel sulphate**, the studies show that oral exposure (in feed and drinking water) at low doses (7.6-11.2 mg Ni/kg bw/day) induces relatively mild effects such as a small decrease in body weight and increased urinary albumin, and at higher doses (25-188 mg Ni/kg bw/day) causes more serious effects such as marked weight loss, atrophy of the thymus and mild tubular nephrosis. The 2-year studies by Ambrose et al. are the most relevant for human lifetime exposure, but are not the most sensitive studies, as effects were found at lower doses in the studies of shorter duration. The Obone et al. 13-week rat study shows a small body weight reduction at a lower dose level than in the 2-year studies (11.2 mg Ni/kg bw/day), and the Vyskocil et al. (1994) 3-6 month study shows increased urinary albumin at approximately the same dose level as the 13-week rat study (7.6 mg Ni/kg bw/day). In agreement with the Obone et al. study, the SLI draft (2002) 90-day oral gavage study in rats showed 8% body weight reduction at 7-11 mg Ni/kg bw/day. In a 2-year OECD TG 451 carcinogenicity study, decreased body weight gain ranging from 4% to 12% was recorded (males and females combined) following oral gavage of 2.2 to 11 mg Ni/kg bw/day. A dose-related reduced survival achieving statistical significance at the two highest dose levels was seen in females. No non-neoplastic microscopic findings, which could be attributed to administration of the test substance, were observed (CRL 2005).
For repeated dose oral toxicity, a LOAEL of 6.7 mg Ni/kg bw/day is set based on reduced body weight and increased mortality (based on CRL 2005). From the same study a NOAEL of 2.2 mg Ni/kg bw/day is set. However, uncertainties remain whether this should actually be considered as a NOAEL, as reduced body weight gain (both sexes) and increased mortality (females) occurred to a statistically non-significant extent.

For **nickel chloride**, two relevant studies with rats are available; both of these studies are suffering from various limitations. The most comprehensive study was a 91-day gavage study, which however suffered from a high mortality and a high number of fatalities caused by gavage errors. In addition to mortality, the animals exhibited salivation, lethargy, and irregular breathing, and decreased body weight. The LOAEL was 5 mg Ni/kg bw/day based on mortality, the lowest dose level in the study. In a 25-week drinking water study, no toxicity was observed at the single dose level studied, 10.2 mg Ni/kg bw/day, however, only a limited number of endpoints were studied. None of these studies are considered adequate for the determination of a NOAEL.

No data for **nickel nitrate** have been located.

6.6.5 Toxicity to reproduction

Reproductive toxicity studies with oral exposure to nickel sulphate and nickel chloride are summarised in Table 7 (fertility studies) and Table 8 (developmental toxicity studies).

Two oral multi-generation reproduction studies and a range-finding one-generation study of **nickel sulphate** in rats are available. No effects on fertility have been observed in these studies at dose levels up to 52-80 mg Ni/kg bw/day.
Effects on male sex organs in rats and mice have been reported in limited studies after oral administration of nickel sulphate from 5.6 mg Ni/kg bw/day. No effects on male sex organs including sperm quality were found in a recent oral OECD TG 416 two-generation study at the highest dose studied, i.e. 2.2 mg Ni/kg bw/day.
The multi-generation studies and the one-generation range-finding study provide consistent evidence of developmental toxicity (stillbirth, post-implantation / peri-natal death) in rats at dose levels not causing maternal toxicity (2.2 mg Ni/kg bw/day).

An oral one-generation study with two successive gestation periods and an oral 2-generation reproduction study of **nickel chloride** in rats are available; no effects on fertility have been found in these studies at dose levels up to 42 mg Ni/kg bw/day.
An increase in abnormalities was observed in spermatozoa from mice treated orally with a single dose of nickel chloride (43 mg Ni/kg bw). Dose related effects on sperm motility and count as well as decreased body weight gain were observed after repeated dosing of mice with nickel chloride at 10 and 20 mg/kg bw/day, but not at a dose level of 5 mg/kg bw/day.
The one- and two-generation studies provide consistent evidence of developmental toxicity (post-implantation / peri-natal death) in rats at dose levels from 1.33 mg Ni/kg bw/day.

No relevant studies regarding **nickel nitrate** have been found.

6.6.6 Mutagenic and genotoxic effects

The mutagenicity and genotoxicity of nickel compounds have been extensively studied.

In general, the nickel compounds gave negative results in bacterial assays with *S. typhimurium* and *E. coli*. Many of the *in vitro* gene mutation studies with mammalian cell lines showed positive results, although these were often weakly positive.
Positive effects have generally been seen in *in vitro* studies of chromosomal effects (chromosomal aberrations, sister chromatid exchanges), cell transformation tests and tests for DNA damage and repair.

The only *in vivo* studies for gene mutations with soluble nickel compounds have been carried out in *Drosophila melanogaster*; weakly positive effects have been seen in one study.
There is evidence that soluble nickel compounds can give rise to both DNA breaks and DNA-protein crosslinks *in vivo*. Recent studies have shown that nickel chloride induced single and double stranded DNA breaks as measured by the Comet assay in leucocytes in mice after oral administration.
Chromosomal aberrations have been seen in a number of studies.

The data from is conflicting. Three micronucleus tests, one of these performed according to the Annex V B12 (OECD 474) protocol, were negative while a number of largely Indian studies have all shown positive results.
The data from dominant lethal tests suggests that there is no significant dominant lethal effect. One study showed a significant increase in sperm head anomalies.

6.6.7 Carcinogenic effects

The carcinogenicity of nickel sulphate following oral administration has been studied in two old non-guideline studies with rats and dogs; no neoplasms were revealed in either rats or dogs in these studies. A recent 2-year carcinogenicity study with rats performed according to OECD TG 451 did not show any carcinogenic potential of exposure to nickel sulphate following oral (gavage) administration.

No data regarding carcinogenicity of nickel chloride and nickel nitrate following oral administration in experimental animals have been located.

Nickel sulphate and nickel chloride have been tested for promoter activity after oral administration. The results indicate that nickel sulphate and nickel chloride may have a promoting effect in combination with selected initiators.

6.7 Evaluation

All nickel compounds release the biologically active form, the nickel ion (Ni^{2+}), in the environment and in the tissues of organisms. Therefore, toxicological effects can be considered together for these substances to the extent that these effects are directly mediated by the nickel ion.
This evaluation is limited to consider the toxicity of the nickel ion, and thus nickel salts from which the nickel ion can be liberated, as this form is the relevant one in relation to estimation of a health based quality criterion in drinking water. Only information of relevance for the estimation of a health based quality criterion in drinking water for soluble inorganic nickel salts has been considered and included in this evaluation.

The soluble nickel compounds (nickel sulphate, nickel chloride, and nickel nitrate) are absorbed from the gastrointestinal tract following oral intake. The available human data indicate that the absorption of nickel following administration in the drinking water to fasting individuals might be as high as up to about 25-27% and about 1-6% when administered to non-fasting individuals and/or together with (or in close proximity to) a meal.

No relevant human data are available.
The three soluble nickel salts, nickel sulphate, nickel chloride and nickel nitrate have shown moderate to high acute oral toxicity with LD_{50} values in the rat from 175 to 1620 mg compound/kg b.w. (43-330 mg Ni/kg b.w.). A toxic class method study with nickel nitrate hexahydrate showed no mortality or signs of toxic symptoms at 200 mg/kg b.w.

Nickel is a well-known skin sensitiser in humans and respiratory sensitisation (work related asthma) has been reported among workers due to exposure to nickel sulphate. The nickel ion is considered exclusively responsible for the immunological effects of nickel.

Worsening of hand eczema has been observed when nickel was given orally to nickel allergic patients. It is not possible to establish a NOAEL for oral challenge in patients with nickel dermatitis. A LOAEL for acute exposure can be established to 12 µg/kg b.w. based on the study in which 9/20 nickel sensitised women had a worsening of their hand eczema following nickel administration on an empty stomach. It should be noted that a LOAEL after repeated exposure may be lower and a LOAEL in non-fasting patients is probably higher because of reduced absorption of nickel ions when mixed in food. On the other hand, systemic exposure to nickel orally in individuals without contact allergy to nickel does not result in sensitisation but may result in immunological tolerance, meaning that the individual is unable to develop contact allergy to nickel at subsequent exposures. Nickel sulphate and nickel chloride are skin sensitisers in guinea pigs. No data regarding respiratory sensitisation in animals have been located.

There are no relevant human data for an evaluation of repeated dose toxicity following oral exposure.
For nickel sulphate, oral (feed and drinking water) repeated dose toxicity studies have been conducted in rats, mice and dogs. Mainly non-specific indications of toxicity, such as decreased body weight, have been observed. In addition, reduced survival, increased urinary albumin (indicator of diminished kidney function), mild tubular nephrosis, as well as immuno-suppressive effects have been observed. An oral LOAEL of 6.7 mg Ni/kg bw/day based on reduced body weight and increased mortality and a NOAEL of 2.2 mg Ni/kg bw/day has been identified in a 2 year gavage study (CRL 2005), although uncertainties remain whether this actually should be considered as a NOAEL as reduced body weight gain (both sexes) and increased mortality (females) occurred to a statistically non-significant extent.
For nickel chloride, two relevant studies with rats are available. Reduced survival was also found with gavage administration of nickel chloride. However, when nickel chloride was administered in drinking water at comparable doses no effects were found. Both of these studies are suffering from various limitations and none are considered adequate for the determination of a NOAEL. A LOAEL of 5 mg Ni/kg bw/day based on mortality was identified in the 91-day gavage study (American Biogenics Corporation 1988).
There are no data on effects following repeated oral exposure with nickel nitrate.
The oral NOAEL of 2.2 mg Ni/kg bw/day for nickel sulphate in the 2-year gavage study is considered as a NOAEL for the soluble inorganic nickel salts of relevance for the setting of a health based quality criterion in drinking water.

There are no relevant human data for an evaluation of reproductive toxicity following oral exposure.

No effects on fertility were observed in two oral multi-generation reproduction studies and a range-finding one-generation study of nickel sulphate in rats at the highest dose levels used (up to 52-80 mg Ni/kg bw/day in an old study and up to 2.2 mg Ni/kg bw/day in a recent OECD TG 416 two-generation study). As the older study has a limited reporting of data, the most reliable NOAEL for fertility effects of nickel sulphate is that from the recent OECD TG 416 two-generation study, i.e. 2.2 mg Ni/kg bw/day.
No effects on fertility were observed in an oral one-generation study and an oral 2-generation reproduction study of nickel chloride in rats at the highest dose levels used (up to 42 mg Ni/kg bw/day). Effects on sperm and oestrus cyclicity were not investigated in these studies. Dose related effects on sperm motility and count as well as decreased body weight gain were observed in mice after repeated dosing with nickel chloride at 10 and 20 mg/kg bw/day, but not at a dose level of 5 mg/kg bw/day; however, due to the limited number of animals used in this study, the dose level of 5 mg/kg bw/day cannot be considered as a reliable NOAEL.

No relevant studies regarding nickel nitrate have been found.

Effects on male sex organs in rats and mice have been reported in limited studies after oral administration of nickel sulphate indicating a LOAEL of 5.6 mg Ni/kg bw/day. No effects on male sex organs including sperm quality were observed in the recent OECD TG 416 two-generation study. The NOAEL for effects on male sex organs is therefore 2.2 mg Ni/kg bw/day.
No relevant studies regarding nickel chloride and nickel nitrate have been found.

No standard prenatal developmental toxicity studies with oral administration were located.
Consistent evidence of developmental toxicity has been seen in rats in the two oral multi-generation studies and the one-generation range-finding with nickel sulphate (stillbirth, post-implantation / peri-natal death) at dose levels not causing maternal toxicity (2.2 mg Ni/kg bw/day). Based on the increased post-implantation / peri-natal lethality in the F_1 generation in the OECD TG 416 two-generation study at 2.2 mg Ni/kg bw/day, the NOAEL is set to 1.1 mg Ni/kg bw/day.
Consistent evidence of developmental toxicity (post-implantation / peri-natal death) was also seen in rats in the one- and two-generation studies with nickel chloride, but a reliable NOAEL cannot be set based on these studies. As all three measures of pup death were statistically significant or borderline significant at the low dose in the second generation in the two-generation study, an equivocal LOAEL for this study was 1.3 mg Ni/kg bw/day.
No relevant studies regarding nickel nitrate have been found.
Based on the OECD TG 416 two-generation study on nickel sulphate, a NOAEL of 1.1 mg Ni/kg bw/day is set for developmental toxicity for the soluble inorganic nickel salts of relevance for the setting of a health based quality criterion in drinking water.

The mutagenicity and genotoxicity of nickel compounds have been extensively studied.
The overall evidence indicates that nickel compounds are not mutagenic in bacteria; however, many of the gene mutation studies with mammalian cell lines showed positive results, although these were often weakly positive. There is considerable evidence for the *in vitro* genotoxicity of nickel compounds with positive effects generally seen in studies of chromosomal effects (chromosomal aberrations, sister chromatid exchanges), cell transformation and of DNA damage and repair.
Interpretation of the results of *in vivo* studies is more complicated. The only *in vivo* studies for gene mutations with soluble nickel compounds have been carried out in *Drosophila melanogaster*; weakly positive effects have been seen in one study. This is consistent with the data seen *in vitro*.
There is evidence that soluble nickel compounds can give rise to both DNA breaks and DNA-protein crosslinks *in vivo*. Chromosomal aberrations have been seen in a number of studies. The data from micronucleus tests is conflicting; however the most valid studies, one of these performed according to the Annex V B12 (OECD 474) protocol, were negative. There are no definitive tests of nickel compounds on the germ cells and there is little evidence for inheritable effects on the germ cells. However, there is evidence that the nickel ion reaches the testis, so a possible effect cannot be excluded. The *in vivo* data thus confirm the clastogenicity seen *in vitro*.
Nickel sulphate, nickel chloride and nickel nitrate are classified as Muta. Cat. 3;R68 based on evidence of *in vivo* genotoxicity in somatic cells after systemic exposure.

There are no human data for an evaluation of carcinogenicity following oral exposure.

With the exception of nickel sulphate, the data on the carcinogenicity of nickel compounds in experimental animals following exposure by oral administration are very limited. A recent 2-year carcinogenicity study with rats performed according to OECD TG 451 did not show any carcinogenic potential of exposure to nickel sulphate following oral (gavage) administration. Similar results were also seen in two old non-guideline studies with rats and dogs.
In conclusion, based on the available data on nickel sulphate, the water soluble nickel compounds are not considered as being carcinogenic following oral administration on their own, although the available data is limited. However, studies evaluating the promoting effect of nickel sulphate and nickel chloride in experimental animals indicate that soluble nickel compounds may have a promoting effect in combination with selected initiators.
It should be noted that nickel compounds are considered as human respiratory carcinogens following inhalation based on epidemiological studies, mechanistic information and evidence from animal studies.

6.7.1 Critical effect and NOAEL

The critical effects following exposure to soluble inorganic nickel salts in drinking water are considered to be sensitisation and developmental toxicity.

Worsening of hand eczema has been observed when nickel was given orally to nickel allergic patients. It is not possible to establish a NOAEL for oral challenge in patients with nickel dermatitis. A LOAEL for acute exposure can be established to 12 µg/kg bw based on the study in which 9/20 nickel sensitised women had a worsening of their hand eczema following nickel administration on an empty stomach. It should be noted that a LOAEL after repeated exposure may be lower and a LOAEL in non-fasting patients is probably higher because of reduced absorption of nickel ions when mixed in food. On the other hand, systemic exposure to nickel orally in individuals without contact allergy to nickel does not result in sensitisation but may result in immunological tolerance, meaning that the individual is unable to develop contact allergy to nickel at subsequent exposures. A health based quality criterion for acute exposure will be estimated based on the LOAEL of 12 µg/kg bw.

There are no relevant human data for an evaluation of reproductive toxicity following oral exposure.
In experimental animals (rats), no effects on fertility and no effects on male sex organs including sperm quality were observed in the recent OECD TG 416 two-generation study at dose levels up to 2.2 mg Ni/kg bw/day (nickel sulphate hexahydrate) and this dose level is thus considered as a NOAEL for effects on fertility and male sex organs. This NOAEL is also the NOAEL for parental toxicity in the OECD TG 416 two-generation study as no effects on growth and no treatment-related clinical signs of toxicity or histopathological changes in the examined organs and tissues were observed in this study. However, it should be noted that based on the 2-year oral OECD TG 451 study (CRL 2005), a NOAEL of 2.2 mg Ni/kg bw/day is set but that uncertainties remain whether this should actually be considered as a NOAEL, as reduced body weight gain (both sexes) and increased mortality (females) occurred to a statistically non-significant extent.
Consistent evidence of developmental toxicity has been seen in rats in the one-, two-, and multi-generation studies with nickel sulphate (stillbirth, post-implantation / peri-natal death) and nickel chloride (post-implantation / peri-natal death) at dose levels not causing maternal toxicity. Based on the increased post-implantation / peri-natal lethality in the F_1 generation in the recent OECD TG 416

two-generation study at 2.2 mg Ni/kg bw/day, the NOAEL for developmental toxicity is set to 1.1 mg Ni/kg bw/day.
In conclusion, a NOAEL of 1.1 mg Ni/kg bw/day is set for developmental toxicity for the soluble inorganic nickel salts of relevance for the setting of a health based quality criterion in drinking water.

A health based quality criterion in drinking water for repeated exposure to soluble inorganic nickel salts will be estimated based on the NOAEL of 1.1 mg Ni/kg bw/day from the OECD TG 416 two-generation study with nickel sulphate. This NOAEL will take into account the uncertainties in the NOAEL of 2.2 mg Ni/kg bw/day from the 2-year oral OECD TG 451 study in which reduced body weight gain (both sexes) and increased mortality (females) occurred to a statistically non-significant extent at the dose level of 2.2 mg Ni/kg bw/day.

According to the report "Humans exposed indirectly via the environment and combined exposure – exposure assessment and risk characterization" (RAR MvE 2008), the NOAEL derived from the OECD TG 416 two-generation study is considered the most relevant to be used for the risk characterisation for repeated dose toxicity for children (1-2 years) instead of the NOAEL from the chronic OECD TG 451 toxicity study for the following reasons.
1) In the chronic toxicity study in rats (CRL 2005), exposure to nickel sulphate began at 6 weeks of age (biologically equivalent to approximately 8-10 years of age in humans) and continued until 104 weeks (end of life, equivalent to 70 years of age in humans). In this study, the animals were not exposed during the time period equivalent to the first one or two years of life in children (approximately weeks 2-3 in rats).
2) In the OECD TG 416 two-generation study (SLI 2000b), exposure to nickel sulphate began *in utero* (from mothers exposed to the same levels through gavage) and continued through lactation up to weaning on postnatal day 21, and then by gavage from weaning until adulthood and through the mating period (for 24 weeks, biologically equivalent to 25-35 years of age in humans), thus covering the first one or two years of children life.
As an alternative to scenario 1, a health based quality criterion in drinking water for repeated exposure to soluble inorganic nickel salts will be estimated based on the NOAEL of 2.2 mg Ni/kg bw/day from the OECD TG 416 two-generation study with nickel sulphate (child-specific approach, included in Appendix 3).

7 TDI and quality criterion

7.1 TDI

The TDI is calculated based on the NOAEL of 1.1 mg Ni/kg bw/day for developmental toxicity in the OECD TG 416 two-generation study (SLI 2000b) with nickel sulphate:

$$TDI = \frac{NOAEL}{UF_I * UF_{II} * UF_{III}} = \frac{1.1 \text{ mg Ni/kg b.w./day}}{10 * 10 * 2} = 5.5 \text{ µg Ni/kg b.w./day}$$

The uncertainty factor UF_I accounting for interspecies variability is set to 10 assuming that humans are more sensitive than animals. The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 2 in order to consider the severity of effects (peri- and postnatal increased mortality) at only twice the dose level of the NOAEL value; a higher factor is not warranted as the NOAEL has been set based on a recent well-performed OECD TG 416 two-generation study.

7.2 Allocation

Human exposure to nickel can result from inhalation, consumption of food and drinking water, or incidental ingestion of soil or dust contaminated with nickel. Table 4 (section 1.4) summarises the estimated exposures from the various media.

Based on the estimated exposures from various media (see Table 4, Section 1.4), it is evident that food is the predominant source of exposure to nickel for both adults and young children. For young children, the exposure from drinking water is about 80 times lower than the exposure from food for the average scenario (or about 1.3% of the exposure from food) and about 12 times lower for the high exposure scenario (or about 8.5% of the exposure from food). For adults, the exposure from drinking water is about 40 times lower than the exposure from food for the average scenario (or about 2.4% of the exposure from food) and about 12 times lower for the high exposure scenario (or about 8.4% of the exposure from food).

The above figures for the nickel exposure from drinking water are based on the level of nickel in groundwater (see Section 1.4). However, when the exposure of nickel in drinking water due to migration of nickel from the different in-house pipes and fittings is added to the exposure from drinking water as estimated above, the contribution from drinking water may be more pronounced. Furthermore, nickel from drinking water is absorbed to a greater extent on an empty stomach than nickel from food, which is reflected in the EU MvE report (2008) where an absorption factor of 0.3 for drinking water and a factor of 0.05 for food are used in the risk assessment.
Because of the higher absorption of nickel from drinking water compared to the absorption from food, allocation of a certain percentage of the external TDI value to drinking water will count up to 6 times more in terms of internal dose, compared

to the internal nickel contribution from food, which will be relatively lower because of the lower absorption.
Therefore, it is not considered appropriate to allocate a greater fraction than 10% of the TDI to drinking water, which also is the general default allocation factor used by the Danish EPA.

An overview of the external and internal indirect environmental nickel exposure as estimated in the report "Humans exposed indirectly via the environment and combined exposure – exposure assessment and risk characterization" (RAR MvE 2008), is presented in Appendix 1.

7.3 Quality criterion in drinking water

7.3.1 Scenarie 1) General population

A health based quality criterion for repeated exposure to soluble inorganic nickel salts is calculated based on the TDI of 5.5 µg Ni/kg b.w./day (based on the NOAEL of 1.1 mg Ni/kg bw/day for developmental toxicity in the OECD TG 416 two-generation study (SLI 2000b) with nickel sulphate)) and assuming a daily ingestion of 2.3 litre of drinking water per day (90th percentile for adults – the 90th percentile is chosen in order to take into account that pregnant women for short periods may have a higher intake of drinking water than generally and because there is a critical window around the time of delivery for the critical effect (postimplantation loss / peri-natal death), i.e., can occur after short-term exposure, body weight: 70 kg) and only 10% of the TDI is allocated to exposure from drinking water (Y):

$$QC_{dw} = \frac{TDI * Y}{ingestion_{dw}} = \frac{5.5\ \mu g\ Ni/kg\ bw/day * 70\ kg * 0.1}{2.3\ l/day}$$

$$= 17\ \mu g\ Ni/l$$

7.3.2 Scenarie 2) Nickel sensitised individuals

Individuals with severe nickel sensitisation are at risk of developing symptoms after oral challenge to nickel in drinking water on an empty stomach. Less sensitive nickel allergic individuals and non-allergic individuals do not experience allergic symptoms after oral nickel intake. A health based quality criterion for acute exposure (worsening of allergic symptoms is considered as an acute event, which can occur after a single exposure) will be estimated based on the LOAEL of 12 µg/kg bw for oral challenge of nickel sensitised individuals and assuming an ingestion of 2.3 litres of drinking water per day (90th percentile for adults, body weight: 70 kg).

$$QC_{dw} = \frac{LOAEL}{UF_{(I*II*III)} * ingestion_{dw}} = \frac{12\ \mu g\ Ni/kg\ bw/day * 70\ kg}{1 * 1 * 10 * 2.3\ l//day}$$

$$= 37\ \mu g\ Ni/l$$

The uncertainty factor UF_I is set to 1 as human data are used. The UF_{II} is set to 1 as the LOAEL has been set for individuals with severe nickel sensitisation, i.e. the most sensitive individuals in the human population. The UF_{III} is set to 10 because a LOAEL instead of a NOAEL is used and because the LOAEL would probably have been lower if the nickel status of the patients was not lowered by giving them a nickel poor diet during the last 2 days before the provocation test.

A quality criterion of 17 μg Ni/l has been calculated based on repeated intake of drinking water and of 37 μg Ni/l based on acute intake of drinking water on an empty stomach.
A health based quality criterion for soluble inorganic nickel compounds of 20 μg Ni/l is proposed. In Appendix 3, further justification for the quality criterion is provided.

This value complies with the present limit value of 20 μg Ni/l (MM 2007).

7.3.3 Quality criterion in drinking water

20 μg Ni/l.

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Appendix 1

External and internal indirect environmental nickel exposure:

An overview of the ***external indirect environmental nickel exposure*** as estimated in the report "Humans exposed indirectly via the environment and combined exposure – exposure assessment and risk characterization" (RAR MvE 2008) is presented for ***adults*** in ***Table 22*** and for ***children*** (1-2 years) in ***Table 23***.

Both ***typical*** (average) exposure and ***reasonable worst case*** (RWC) exposures (corresponding to 95-percentiles) are included in the tables, which cover both regional scenarios representing background levels as well as local emission scenarios from different kind of industrial nickel sectors.

Exposure expressed per kg body weight for adults takes into account a body weight of 70 kg and for children of 1-2 years a body weight of 11.5 kg. For both the regional and local scenarios, dietary exposure seems to be the dominant pathway (> 95 % for the regional scenario and > 75 % for the local scenarios).

Table 24 and 25 further contain information on the ***internal exposure*** as the external exposures presented in Tables 22 and 23 have been converted to the corresponding internal exposures. For calculation of the internal exposures, an absorption factor of 0.5 has been used for inhalation exposure, while oral absorption factors of 0.3 and 0.05 have been used in relation to intake from drinking water and food, respectively. (RAR MvE 2008)

Table 22: overview of external Ni exposure to adults via the various pathways (upper part: typical scenarios; lower part: RWC scenarios)

	TYPICAL										
Scenario	AIR			SOIL/DUST			WATER			FOOD	
	conc	external dose		conc	external dose[a]		conc	external dose		external dose	
	µg/m³	µg/day	µg/day.kg bw	mg/kg	µg/day	µg/day.kg bw	µg/l	µg/day	µg /day.kg bw	µg/day	µg /day.kg bw
REGIONAL											
standard scenario: tap water	0.0045	0.09	0.001	19.2 (soil)/ 49 (dust)	1.8	0.025	1.5	2.0	0.028	115	1.7
Additional scenario: groundwater	0.0045	0.09	0.001	19.2 (soil)/ 49 (dust)	1.8	0.025	2.0	2.6	0.037	115	1.7
high Ni diet scenario[d]	0.0045	0.09	0.001	19.2 (soil)/ 49 (dust)	1.8	0.025				352	5.0
LOCAL											
Refining	2.5	50	0.72	28.9 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	169	2.4
stainless steel	0.42	8.4	0.12	31.6 (soil)[b]	2.0	0.029	1.5[c]	2.0	0.028	120	1.7
multiple steel	0.08	1.6	0.023	27.8 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	117	1.7
Ni alloy	0.03	0.60	0.009	27.8 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	116	1.7
steel production	0.07	1.4	0.020	27.9 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	117	1.7
Chemicals	0.06	1.2	0.017	31.6 (soil)[b]	2.1	0.029	1.5[c]	2.0	0.028	117	1.7
Catalysts	0.02	0.40	0.006	27.8 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	116	1.7
Plating	0.00	0.04	0.001	28.0 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	117	1.7
metal products	0.00	0.08	0.001	31.6 (soil)[b]	2.1	0.029	1.5[c]	2.0	0.028	115	1.7
Batteries	0.01	0.20	0.003	31.6 (soil)[b]	2.1	0.029	1.5[c]	2.0	0.028	115	1.7
powder metallurgy	0.00	0.08	0.001	27.8 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	115	1.7
Recycling	0.00	0.06	0.001	27.9 (soil)[b]	2.0	0.028	1.5[c]	2.0	0.028	116	1.7

a external dose includes both dust and soil ingestion (ingestion of 22.5 mg/day soil and 27.5 mg/day indoor dust for adults)

b indoor dust component is not local sector-specific considered (regional values applicable)

c drinking water is not local scale specific (regional values are applicable)

d based on 99th percentile Ni dietary intake; this dietary intake included water and other beverages intake; double counting with the water exposure route should be avoided

	RWC										
Scenario	AIR			SOIL/DUST			WATER			FOOD	
	conc	external dose		conc	external dose		conc	external dose		external dose	
		µg/day	µg /day.kg bw		µg/day	µg /day.kg bw		µg/day	µg /day.kg bw	µg/day	µg /day.kg bw
REGIONAL											
standard scenario: tap water	0.015	0.29	0.004	26.2 (soil)/ 255 (dust)	7.6	0.11	3.7	8.5	0.12	239	3.4
additional scenario: groundwater	0.015	0.29	0.004	26.2 (soil)/ 255 (dust)	7.6	0.11	8.8	20.2	0.29	239	3.4
high Ni diet scenario											
LOCAL											
Refining	3.6	71	1.0	29.4 (soil)[b]	7.7	0.11	3.7[c]	8.5	0.12	316	4.5
stainless steel	1.6	32	0.46	33.4 (soil)[b]	7.8	0.11	3.7[c]	8.5	0.12	360	5.2
multiple steel	0.18	3.6	0.051	27.9 (soil)[b]	7.6	0.11	3.7[c]	8.5	0.12	242	3.5
Ni alloy	0.31	6.2	0.089	28.4 (soil)[b]	7.7	0.11	3.7[c]	8.5	0.12	242	3.5
steel production	0.26	5.2	0.074	32.1 (soil)[b]	7.7	0.11	3.7[c]	8.5	0.12	345	4.9
Chemicals	0.32	6.4	0.091	33.0 (soil)[b]	7.8	0.11	3.7[c]	8.5	0.12	246	3.5
Catalysts	0.11	2.2	0.031	31.7 (soil)[b]	7.7	0.11	3.7[c]	8.5	0.12	252	3.6
Plating	0.01	0.20	0.003	32.0 (soil)[b]	7.7	0.11	3.7[c]	8.5	0.12	332	4.7
metal products	0.01	0.10	0.001	31.7 (soil)[b]	7.7	0.11	3.7[c]	8.5	0.12	240	3.4
batteries	0.02	0.40	0.006	32.7 (soil)[b]	7.8	0.11	3.7[c]	8.5	0.12	307	4.4
powder metallurgy	0.02	0.40	0.006	27.8 (soil)[b]	7.6	0.11	3.7[c]	8.5	0.12	239	3.4
recycling	0.00	0.06	0.001	31.0 (soil)[b]	7.7	0.11	3.7[c]	8.5	0.12	240	3.4

[a] external dose includes both dust and soil ingestion (ingestion of 22.5 mg/day soil and 27.5 mg/day indoor dust for adults)

[b] indoor dust component is not local sector-specific considered (regional values applicable) [c] drinking water is not local scale specific (regional values are applicable)

Table 23: overview of external Ni exposure to children (1-2 years) via the various pathways (upper part: typical scenarios; lower part: RWC scenarios)

	TYPICAL										
scenario	AIR			SOIL/DUST			WATER			FOOD	
	conc	external dose		Conc	external dose[a]		conc	external dose		external dose	
	µg/m³	µg/day	µg / day.kg bw	mg/kg	µg/day	µg / day.kg bw	µg/l	µg/day	µg / day.kg bw	µg/day	µg / day.kg bw
REGIONAL											
standard scenario: tap water	0.0045	0.05	0.0039	19.2 (soil)/ 49 (dust)	4.8	0.42	1.50	0.75	0.065	63	5.5
additional scenario: groundwater	0.0045	0.05	0.0039	19.2 (soil)/ 49 (dust)	4.8	0.42	2.00	1.00	0.087	63	5.5
LOCAL											
refining	2.5	25	2.2	28.9 (soil)[b]	5.4	0.47	1.5[c]	0.75	0.065	73	6.4
stainless steel	0.42	4.2	0.37	31.6 (soil)[b]	5.5	0.48	1.5[c]	0.75	0.065	64	5.6
multiple steel	0.08	0.80	0.070	27.8 (soil)[b]	5.3	0.46	1.5[c]	0.75	0.065	63	5.5
Ni alloy	0.03	0.30	0.026	27.8 (soil)[b]	5.3	0.46	1.5[c]	0.75	0.065	63	5.5
steel production	0.07	0.70	0.061	27.9 (soil)[b]	5.3	0.46	1.5[c]	0.75	0.065	63	5.5
chemicals	0.06	0.60	0.052	31.6 (soil)[b]	5.5	0.48	1.5[c]	0.75	0.065	63	5.5
catalysts	0.02	0.20	0.017	27.8 (soil)[b]	5.3	0.46	1.5[c]	0.75	0.065	63	5.5
plating	0.00	0.02	0.002	28.0 (soil)[b]	5.3	0.46	1.5[c]	0.75	0.065	63	5.5
metal products	0.00	0.04	0.003	31.6 (soil)[b]	5.5	0.48	1.5[c]	0.75	0.065	63	5.5
batteries	0.01	0.10	0.009	31.6 (soil)[b]	5.5	0.48	1.5[c]	0.75	0.065	63	5.5
powder metallurgy	0.00	0.04	0.003	27.8 (soil)[b]	5.3	0.46	1.5[c]	0.75	0.065	63	5.5
recycling	0.00	0.03	0.003	27.9 (soil)[b]	5.3	0.46	1.5[c]	0.75	0.065	63	5.5

[a] external dose includes both dust and soil ingestion (ingestion of 61 mg/day soil and 74 mg/day indoor dust for children)

[b] indoor dust component is not local sector-specific considered (regional values applicable) [c] drinking water is not local scale specific (regional values are applicable)

	RWC										
scenario	AIR			SOIL/DUST			WATER			FOOD	
	conc	external dose		Conc	external dose[a]		conc	external dose		external dose	
	µg/m³	µg/day	µg/ day.kg bw	mg/kg	µg/day	µg /day.kg bw	µg/l	µg/day	µg / day.kg bw	µg/day	µg / day.kg bw
REGIONAL											
standard scenario: tap water	0.0145	0.145	0.013	26.2 (soil)/ 255 (dust)	21	1.8	3.7	2.3	0.20	107	9.3
additional scenario: groundwater	0.015	0.145	0.013	26.2 (soil)/ 255 (dust)	21	1.8	8.8	5.5	0.48	107	9.3
LOCAL											
refining											
stainless steel	3.6	36	3.1	29.4 (soil)[b]	21	1.8	3.7c	2.3	0.20	122	11
multiple steel	1.6	16	1.4	33.4 (soil)[b]	21	1.8	3.7c	2.3	0.20	130	11.
Ni alloy	0.18	1.8	0.16	27.9 (soil)[b]	21	1.8	3.7c	2.3	0.20	108	9.4
steel production	0.31	3.1	0.27	28.4 (soil)[b]	21	1.8	3.7c	2.3	0.20	108	9.4
chemicals	0.26	2.6	0.23	32.1 (soil)b	21	1.8	3.7c	2.3	0.20	127	11
catalysts	0.32	3.2	0.28	33.0 (soil)[b]	21	1.8	3.7c	2.3	0.20	108	9.4
plating	0.11	1.1	0.096	31.7 (soil)[b]	21	1.8	3.7c	2.3	0.20	110	9.6
metal products	0.01	0.10	0.009	32.0 (soil)[b]	21	1.8	3.7c	2.3	0.20	125	11
batteries	0.01	0.05	0.004	31.7 (soil)[b]	21	1.8	3.7c	2.3	0.20	107	9.3
powder metallurgy	0.02	0.20	0.017	32.7 (soil)[b]	21	1.8	3.7c	2.3	0.20	120	10
recycling	0.02	0.20	0.017	27.8 (soil)[b]	21	1.80	3.7c	2.3	0.20	107	9.3

[a] external dose includes both dust and soil ingestion (ingestion of 61 mg/day soil and 74 mg/day indoor dust for children)

[b] indoor dust component is not local sector-specific considered (regional values applicable)

[c] drinking water is not local scale specific (regional values are applicable)

Table 24: Summary of indirect exposures to and absorbed doses of Ni via the environment for adults (upper table: typical scenarios; lower table: RWC scenarios).

	TYPICAL															
Scenario	AIR				SOIL/DUST				WATER				FOOD			
	external exposure	internal dose	internal dose	% total intake	external exposure	internal dose	internal dose	% total intake	external exposure	internal dose	internal dose	% total intake	external exposure	internal dose	internal dose	% total intake
	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%
REGIONAL																
general tap water scenario	0.09	0.045	0.0006	0.7%	1.8	0.09	0.0013	1.4%	2.0	0.59	0.008	9.0%	115	5.8	0.082	89%
Danish groundwater scenario	0.09	0.045	0.0006	0.7%	1.8	0.09	0.0013	1.3%	2.6	0.78	0.011	11.7%	115	5.8	0.082	86%
high Ni diet scenario[a]	0.09	0.045	0.0006	0.1%	1.8	0.09	0.0013	0.2%					352	58.1	0.83	99%
LOCAL																
Refining	50	25	0.36	73%	2.0	0.10	0.0014	0.3%	2.0	0.59	0.008	1.7%	169	8.4	0.12	25%
stainless steel	8.4	4.2	0.06	39%	2.1	0.10	0.0015	0.9%	2.0	0.59	0.008	5.4%	120	6.0	0.086	55%
multiple steel	1.6	0.8	0.011	11%	2.0	0.10	0.0014	1.3%	2.0	0.59	0.008	8.0%	117	5.8	0.083	80%
Ni alloy	0.60	0.3	0.0043	4.4%	2.0	0.10	0.0014	1.5%	2.0	0.59	0.008	8.6%	116	5.8	0.083	85%
steel production	1.4	0.7	0.010	9.7%	2.0	0.10	0.0014	1.4%	2.0	0.59	0.008	8.1%	117	5.9	0.084	81%
Chemicals	1.2	0.6	0.0086	8.4%	2.1	0.10	0.0015	1.4%	2.0	0.59	0.008	8.2%	117	5.8	0.083	82%
Catalysts	0.40	0.2	0.0029	3.0%	2.0	0.10	0.0014	1.5%	2.0	0.59	0.008	8.8%	116	5.8	0.083	87%
Plating	0.04	0.02	0.0003	0.3%	2.0	0.10	0.0014	1.5%	2.0	0.59	0.008	8.9%	117	5.8	0.083	89%
metal products	0.08	0.04	0.0006	0.6%	2.1	0.10	0.0015	1.6%	2.0	0.59	0.008	9.0%	115	5.8	0.082	89%
Batteries	0.20	0.1	0.0014	1.5%	2.1	0.10	0.0015	1.6%	2.0	0.59	0.008	8.9%	115	5.8	0.082	88%
powder metallurgy	0.08	0.04	0.0006	0.6%	2.0	0.10	0.0014	1.5%	2.0	0.59	0.008	9.0%	115	5.8	0.082	89%
Recycling	0.06	0.03	0.0004	0.5%	2.0	0.10	0.0014	1.5%	2.0	0.59	0.008	9.0%	116	5.8	0.083	89%

	RWC															
Scenario	AIR				SOIL/DUST				WATER				FOOD			
	External dose	internal dose	internal dose	% total intake	external dose	internal dose	internal dose	% total intake	external dose	internal dose	internal dose	% total intake	external dose	internal dose	internal dose	% total intake
	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/l	%	µg/day	µg/day	µg/day	%
REGIONAL																
standard scenario: tapwater	0.29	0.15	0.002	1.0%	1.77	0.089	0.0054	2.5%	8.5	2.6	0.036	17%	239	12	0.17	79%
additional scenario: groundwater	0.29	0.15	0.002	0.8%	1.77	0.089	0.0054	2.1%	20	6.07	0.087	33%	239	12	0.17	64%
LOCAL																
Refining	71	36	0.51	65%	1.99	0.099	0.0055	0.7%	8.5	2.6	0.036	4.7%	316	16	0.23	29%
stainless steel	32	16	0.23	43%	2.05	0.103	0.0055	1.1%	8.5	2.6	0.036	6.9%	360	18	0.26	49%
multiple steel	3.6	1.8	0.026	11%	1.96	0.098	0.0055	2.3%	8.5	2.6	0.036	15%	242	12	0.17	72%
Ni alloy	6.2	3.1	0.044	17%	1.96	0.098	0.0055	2.1%	8.5	2.6	0.036	14%	242	12	0.17	67%
steel production	5.2	2.6	0.037	11%	1.97	0.098	0.0055	1.7%	8.5	2.6	0.036	11%	345	17	0.25	76%
Chemicals	6.4	3.2	0.046	17%	2.05	0.103	0.0055	2.1%	8.5	2.6	0.036	14%	246	12	0.18	67%
Catalysts	2.2	1.1	0.016	6.6%	1.96	0.098	0.0055	2.3%	8.5	2.6	0.036	15%	252	13	0.18	76%
Plating	0.20	0.10	0.001	0.5%	1.97	0.098	0.0055	2.0%	8.5	2.6	0.036	13%	332	17	0.24	85%
metal products	0.10	0.05	0.001	0.3%	2.05	0.103	0.0055	2.6%	8.5	2.6	0.036	17%	240	12	0.17	80%
Batteries	0.40	0.20	0.003	1.1%	2.05	0.103	0.0055	2.1%	8.5	2.6	0.036	14%	307	15	0.22	83%
powder metallurgy	0.40	0.20	0.003	1.3%	1.96	0.098	0.0055	2.5%	8.5	2.6	0.036	17%	239	12	0.17	79%
Recycling	0.06	0.03	0.000	0.2%	1.97	0.098	0.0055	2.6%	8.5	2.6	0.036	17%	240	12	0.17	80%

[a] based on the 99th percentile Ni dietary intake; water intake is already included in the dietary intake of the high Ni diet scenario; double counting with water as an extra exposure pathway should be avoided.

Table 25: Summary of indirect exposures to and absorbed doses of Ni via the environment for children (1-2 years) (upper table: typical scenarios; lower table: RWC scenarios).

	TYPICAL															
Scenario	AIR				SOIL/DUST				WATER				FOOD			
	external exposure	internal dose	internal dose	% total intake	external exposure	internal dose	internal dose	% total intake	external exposure	internal dose	internal dose	% total intake	external exposure	internal dose	internal dose	% total intake
	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%
REGIONAL																
general tap water scenario	0.05	0.023	0.002	0.6%	4.8	0.24	0.021	6.6%	0.75	0.23	0.02	6.2%	63	3.2	0.27	87%
Danish groundwater scenario	0.05	0.023	0.002	0.6%	4.8	0.24	0.021	6.4%	1.00	0.30	0.03	8.1%	63	3.2	0.27	85%
LOCAL																
Refining	25	13	1.1	75%	5.4	0.27	0.023	1.6%	0.75	0.23	0.02	1.3%	73	3.7	0.32	22%
stainless steel	4.2	2.1	0.18	36%	5.5	0.28	0.024	4.8%	0.75	0.23	0.02	3.9%	64	3.2	0.28	55%
multiple steel	0.80	0.4	0.035	9.9%	5.3	0.27	0.023	6.6%	0.75	0.23	0.02	5.6%	63	3.2	0.27	78%
Ni alloy	0.30	0.15	0.013	4.0%	5.3	0.27	0.023	7.0%	0.75	0.23	0.02	5.9%	63	3.2	0.27	83%
steel production	0.70	0.35	0.030	8.8%	5.3	0.27	0.023	6.7%	0.75	0.23	0.02	5.6%	63	3.2	0.27	79%
Chemicals	0.60	0.3	0.026	7.6%	5.5	0.28	0.024	7.0%	0.75	0.23	0.02	5.7%	63	3.2	0.27	80%
Catalysts	0.20	0.1	0.009	2.7%	5.3	0.27	0.023	7.1%	0.75	0.23	0.02	6.0%	63	3.2	0.27	84%
Plating	0.02	0.01	0.001	0.3%	5.3	0.27	0.023	7.3%	0.75	0.23	0.02	6.2%	63	3.2	0.27	86%
metal products	0.04	0.02	0.002	0.5%	5.5	0.28	0.024	7.5%	0.75	0.23	0.02	6.1%	63	3.2	0.27	86%
Batteries	0.10	0.05	0.004	1.4%	5.5	0.28	0.024	7.5%	0.75	0.23	0.02	6.1%	63	3.2	0.27	85%
powder metallurgy	0.04	0.02	0.002	0.5%	5.3	0.27	0.023	7.2%	0.75	0.23	0.02	6.1%	63	3.2	0.27	86%
Recycling	0.03	0.015	0.001	0.4%	5.3	0.27	0.023	7.3%	0.75	0.23	0.02	6.2%	63	3.2	0.27	86%

	RWC															
Scenario	AIR				SOIL/DUST				WATER				FOOD			
	External dose	internal dose	internal dose	% total intake	external dose	internal dose	internal dose	% total intake	external dose	internal dose	internal dose	% total intake	external dose	internal dose	internal dose	% total intake
	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/day.kg bw	%	µg/day	µg/day	µg/l	%	µg/day	µg/day	µg/day	%
REGIONAL																
standard scenario: tapwater	0.15	0.07	0.006	1%	4.8	0.24	0.09	14%	2.3	0.7	0.06	10%	107	5.4	0.47	75%
additional scenario: groundwater	0.15	0.07	0.006	1%	4.8	0.24	0.09	13%	5.5	1.7	0.14	21%	107	5.4	0.47	66%
LOCAL																
Refining																
stainless steel	36	18	1.5	69%	5.4	0.27	0.09	4%	2.3	0.7	0.06	3%	122	6.1	0.53	24%
multiple steel	16	8.0	0.70	49%	5.5	0.28	0.09	6%	2.3	0.7	0.06	4%	130	6.5	0.57	40%
Ni alloy	1.8	0.90	0.078	11%	5.3	0.27	0.09	13%	2.3	0.7	0.06	9%	108	5.4	0.47	67%
steel production	3.1	1.6	0.14	18%	5.3	0.27	0.09	12%	2.3	0.7	0.06	8%	108	5.4	0.47	62%
Chemicals	2.6	1.3	0.11	14%	5.3	0.27	0.09	11%	2.3	0.7	0.06	7%	127	6.4	0.55	68%
Catalysts	3.2	1.6	0.14	18%	5.5	0.28	0.09	12%	2.3	0.7	0.06	8%	108	5.4	0.47	62%
Plating	1.1	0.55	0.048	7%	5.3	0.27	0.09	13%	2.3	0.7	0.06	9%	110	5.5	0.48	71%
metal products	0.10	0.05	0.004	1%	5.3	0.27	0.09	13%	2.3	0.7	0.06	9%	125	6.3	0.54	78%
Batteries	0.05	0.03	0.002	0%	5.5	0.28	0.09	15%	2.3	0.7	0.06	10%	107	5.4	0.47	75%
powder metallurgy	0.20	0.10	0.009	1%	5.5	0.28	0.09	13%	2.3	0.7	0.06	9%	120	6.0	0.52	76%
Recycling	0.20	0.10	0.009	1%	5.3	0.27	0.09	14%	2.3	0.7	0.06	10%	107	5.4	0.47	75%

Appendix 2

Statistical re-analysis of the 2-generation OECD TG 416 study and NOAEL identification:

A 2-generation reproduction study compliant with the OECD TG 416 has been performed in which Sprague-Dawley rats were administered nickel sulphate hexahydrate at dose levels of 1, 2.5, 5.0, and 10 mg/kg bw/day by gavage (NiPERA 2000b), see section 4.5.1.

The post-implantation / peri-natal lethality until postnatal day 0 among the F_1 offspring (i.e. number of pups conceived minus the number of live pups at birth, see Table 10) was higher at 10 mg/kg bw/day, however, the difference was not statistically significant (2.1 at 10 mg/kg bw/day vs. 0.9 in the control group, p = 8.6% in Mann-Whitney test). In F2 offspring, the value for post-implantation / peri-natal lethality was similar to the F2 control value.

The authors state that the results indicate that the highest dose of 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) was a NOAEL for the developmental end points studied, including the variable of post-implantation / peri-natal lethality. As peri-natal lethality also occurs after the day of birth, the Danish EPA wanted to evaluate the whole time period from implantation to peri-natal day 4 as a continuum to which NiPERA agreed. For the dose group of 2.2 mg Ni/kg bw/day the post-implantation / peri-natal lethality is 2.29+0.43 (mean + sem) per litter and for the control group it is 1.00+0.22 per litter. The statistical analysis gives a p-value of 5.8% in Mann-Whitney test.

When analysing post-implantation loss in prenatal developmental toxicity studies, this parameter is often calculated as percentage lost per litter. A similar calculation for post-implantation / peri-natal lethality until peri-natal day 4 gives a value of 7.1+1.5% (mean + sem) in the control group and 15.8+2.8% in the 2.2 mg Ni/kg bw/day group. This difference is statistically significant in Mann-Whitney test (p = 4.4%).

The data includes several litters with no lethality (11 of 25 in the control, 8 of 28 at 2.2 mg Ni/kg bw/day) and the distributions of data for the two groups do not seem to have the same shape, see Figure 1.

Although data for Mann-Whitney test are not assumed to follow a specific probability distribution, it is assumed that the underlying populations are continuous and have the same shape. Consequently, the Mann-Whitney test may not be the most appropriate test for the present data. Therefore, the data was also analysed by Fisher chi-square. In the control group, 0 of 25 litters had more than 3 losses, while in the 2.2 mg Ni/kg bw/day group 8 of 28 litters (29%) had more than 3 losses (range 4-7). The difference is statistically significant (p-value in Fisher chi-square test is 0.5%). Fisher chi-square test on the number of litters with more than 30% loss (0 of 25 in controls and 8 of 28 at 2.2 mg Ni/kg bw/day) gives a similar result as for the number of losses above, i.e. the p-value is 0.5%.

The results in the first generation of the study were further analysed by Sommer et al. (2002). The data were analysed in a general linear model with

overdispersion and used the litter as the statistical unit. The main result shows a significant raise in the peri-postnatal mortality rate in the group exposed to 2.2 mg Ni/kg bw/day compared to the control group (p = 0.8%) and also when compared to the pooled data from the control group and the exposed groups 2, 3, and 4 (p = 0.04%). As the latter analysis includes values for exposed groups as control values, it may actually underestimate the significance of the increased value for peri-postnatal loss in the group exposed to 2.2 mg Ni/kg bw/day. Historical control group mean values for post-implantation / prenatal loss at day 0 from 8 studies range from 0.9-2.3 per litter. The value of 2.1 per litter for the group exposed to 2.2 mg Ni/kg bw/day is within this range. However, the number of implantations and the number of live pups per litter in the historical controls are generally higher than the values in the 2-generation study of nickel sulphate, see Table 26. Dams with a high ovulation may tend to show higher pre- and post-implantation losses to give normal litter size and therefore the historical control values for loss are not considered as the most relevant for evaluating the loss in the 2-generation study. The concurrent control values for loss appears relevant based on the number of implantation and consequently this value is used for evaluating the loss in the exposed groups.

FIG 1. Post-implantation/Perinatal lethality (until day 4) in the 2-generation study

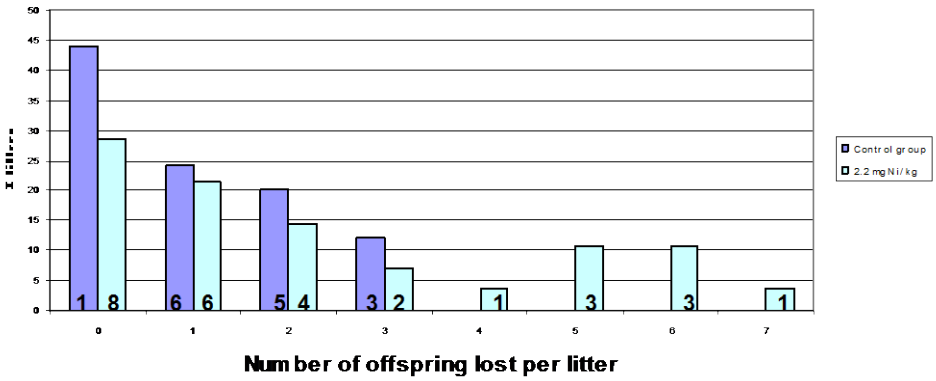

Table 26. Historical control values from 8 studies compared to control group in the NiPERA (2000b) 2-generation study

	Implantations per litter	Live pups per litter	Loss per litter
Historical control values (8 studies)	14.8-17.3	13.0-15.5	0.9-2.3
Control group, 2-generation study	13.6	12.6	0.9

In conclusion, statistical analysis using the litter as the unit of significance shows that there is a statistically significant increase in litters with high post-implantation / peri-natal lethality and in the mean percentage post-implantation / peri-natal lethality in F_1 group dosed with 2.2 mg Ni/kg bw/day.
There was no statistically significant effect on post-implantation / peri-natal lethality in F_2 offspring. However, the parental animals for this generation were selected from the F_1 generation and obviously the F_1 offspring that died pre- or post-natally are not represented. Consequently, the animals that may have had

the highest sensitivity to the effect may not have been included in the production of F_2.
Based on the supplementary statistics using the litter as the statistical unit and showing that the increase in post-implantation / peri-natal lethality in F_1 is statistically significant as well as the above consideration concerning the finding of effects in F_1 but not in F_2, it is evaluated that the 10 mg/kg bw/day (2.2 mg Ni/kg bw/day) cannot be regarded as a clear NOAEL. Consequently, the NOAEL is set to 5 mg/kg bw/day (1.1 mg Ni/kg bw/day) in this study.
Since the highest dose level in this 2-generation study did not induce any signs of toxicity in the P_0 animals, the study does not fulfil the OECD TG 416 concerning the dose levels used. As the results of the prior range-finding one-generation study indicate that post-implantation / peri-natal lethality is increased in the absence of maternal toxicity, it is considered acceptable for the evaluation of developmental toxicity that the highest dose level did not induce maternal toxicity.

Appendix 3

Further justification for the proposed quality criterion:

According to the guidelines for setting health based quality criteria for chemical substances in soil, ambient air and drinking water[1] laid down by the Danish Environmental Protection Agency, a health based quality criterion for soluble inorganic nickel compounds of 20 µg Ni/l has been proposed. This appendix provides further justification for the proposed quality criterion.

The Danish quality criterion and the WHO guideline value

The proposed quality criterion of 20 µg Ni/l is 3.5 times lower than the guideline value of 70 µg Ni/l set by the WHO (WHO 2008). The similarities and differences in the derivation of the quality criterion versus the WHO guideline value are summarised in Table 27, and the most important differences are further addressed in the text.

Table 27. Similarities and differences in the derivation of the Danish quality criterion of 20 µg Ni/l versus the WHO guideline value of 70 µg Ni/l

Parameter	Danish EPA	WHO
Critical effect	Peri- and postnatal mortality	Post-implantation/peri-natal lethality
Study	OECD TG 416 two-generation study (SLI 2000b)	OECD TG 416 two-generation study (SLI 2000b)
NOAEL	1.1 mg Ni/kg bw/day	1.1 mg Ni/kg bw/day
Uncertainty factor	200 (10 x 10 x 2)	100 (10 x 10)
TDI	5.5 µg/kg bw/day	11 µg/kg bw/day
Allocation (% of the TDI)	10	20
Body weight, adult	70 kg	60 kg
Daily intake of water	2.3 litres/day (90th percentile	2 litres/day
Quality criterion / guideline value	20 µg/litre (rounded from 17 µg/litre)	70 µg/litre (rounded from 66 µg/litre)

Critical effect / NOAEL / Assessment factors/ TDI

Both the Danish EPA and the WHO have estimated the TDI based on the NOAEL of 1.1 mg Ni/kg bw/day for developmental toxicity in the OECD TG 416 two-generation study (SLI 2000b) with nickel sulphate.

The Danish EPA estimated a TDI of 5.5 µg/kg bw/day by application of an uncertainty factor of 200 (10 for interspecies variation, 10 for intraspecies variation, and 2 in order to consider the severity of effects (peri- and postnatal

[1] Vejledning fra Miljøstyrelsen, 5/2006 "Metoder til fastsættelse af kvalitetskriterier for kemiske stoffer i jord, luft og drikkevand med henblik på at beskytte sundheden". *In Danish*. http://www.mst.dk/-Udgivelser/-Publikationer/-2006/-08/-87-7052-182-4.htm

mortality) at only twice the dose level of the NOAEL value). This uncertainty factor is in accordance with the reference 'Margin of Safety' (MOSref) of 200-300 (which included a severity factor of 2-3) applied for the endpoint 'Developmental toxicity' in the EU Risk Assessment Reports. This MOSref has been agreed by the Technical Committee for New and Existing Substances.

The WHO estimated a TDI of 11 µg/kg bw/day by application of an uncertainty factor of 100 (10 to account for interspecies variation and 10 to account for intraspecies variation).

Allocation

The Danish EPA has allocated only 10% of the external TDI to intake of nickel from drinking water justified by the estimated daily intake of nickel from various media showing that the diet is far the major source of the external nickel exposure contributing with 90% or more to the daily intake, see also section 7.2.

Furthermore, nickel from drinking water is absorbed to a greater extent on an empty stomach than nickel from food, which is reflected in the EU MvE report (2008) where an absorption factor of 0.3 for drinking water and a factor of 0.05 for food are used in the risk assessment.
Because of the higher absorption of nickel from drinking water compared to the absorption from food, allocation of a certain percentage of the external TDI value to drinking water will count up to 6 times more in terms of internal dose, compared to the internal nickel contribution from food, which will be relatively lower because of the lower absorption.
Therefore, it is not considered appropriate to allocate a greater fraction than 10% of the TDI to drinking water, which also is the general default allocation factor used by the Danish EPA.

The WHO has used an allocation of 20% of the TDI to drinking-water (which is in accordance with the new default value used by WHO in relation to drinking water) and it is indicated that the guideline value of 70 ug/l also will allow for circumstances where naturally elevated nickel occurs in drinking-water.

Implication of the quality criterion of 20 µg Ni/l in relation to the TDI

Intake of drinking water containing nickel at a concentration equal to the proposed quality criterion of 20 µg Ni/l will for adults (body weight: 70 kg) result in an internal dose of 0.12 and 0.20 µg Ni/kg bw/day for an average (mean: 1.3 litre/day) and a high (90^{th} percentile: 2.3 litres/day) intake of drinking water, respectively, based on an absorbed factor of 30% for nickel in drinking water as used in the RAR MvE (2008)

According to the report RAR MvE (2008) report, the internal dose of nickel from the diet has for adults been estimated to 0.082 and 0.17 µg Ni/kg bw/day for the typical and 'reasonable worst case' scenarios, respectively.

The *total* internal exposure of nickel from the *diet* and from *drinking water* containing nickel at a concentration equal to the proposed quality criterion of 20 µg Ni/l is then *0.20* and *0.37 µg Ni/kg bw/day* for the typical and 'reasonable worst case' scenarios, respectively.

A Tolerable Daily Intake (TDI) of 5.5 μg Ni/kg bw/day has been estimated based on the NOAEL of 1.1 mg Ni/kg bw/day for developmental toxicity in the OECD TG 416 two-generation study (SLI 2000b) with nickel sulphate (section 7.1). This TDI corresponds to an internal dose of *0.28 μg Ni/kg bw/day* when using an absorbed factor of 5% in connection with gavage administration of the animals as used in the risk assessment in the RAR MvE (2008).

Thus at a drinking water content of 20 μg Ni/l the total daily internal exposure makes up about 70% and 130% of the TDI (converted to internal value) for the typical and 'reasonable worst case' scenarios in relation to additional exposure through food.

Although this calculation is considered conservative because of the assumption of a high absorption factor from the total amount of water ingested over a day this provides further support that a quality criterion of 20 μg Ni/l is justified.

Thus, exposure at a nickel concentration of 70 μg Ni/l in drinking water (the guideline value proposed by the WHO) would, together with the nickel exposure from food, very clearly exceed the TDI when the exposures are converted to internal exposures by using the above-mentioned absorption factors.

Ad 'Quality criterion in drinking water' (section 7.3)

Child-specific scenario

In the Danish guidance for elaboration of quality criteria, it is recommended to calculate a child specific value in order to assure that children also are protected.
Thus, in addition to the scenarios selected for the calculation of a health based quality criterion in drinking water for acute and repeated dose exposure to nickel in drinking water as presented in section 7.3, a child-specific scenario has also been evaluated.

The NOAEL of 1.1 mg Ni/kg bw/day selected for the estimation of a TDI is related to pre- and peri-natal exposure, i.e., exposure of the foetus / the new born child. This value was in the MvE RAR (2008) not considered adequate for children at later life stages. Concerning repeated exposure for children, a NOAEL of 2.2 mg Ni/kg bw/day was instead concluded based on the OECD TG 416 two-generation study, as no effects on growth and no treatment-related clinical signs of toxicity or histopathological changes in the examined organs and tissues were observed among the offspring surviving the peri-natal period in this study.
Assuming a daily ingestion of 0.03 l/kg bw/day of drinking water (median value for children 1-10 years old) and that only 10% of the daily intake of nickel is allocated to exposure from drinking water (Y), the following quality criterion can be calculated:

$$QC_{dw} = \frac{NOAEL * Y}{UF_{(I*II*III)} * ingestion_{dw}} = \frac{2.2 \text{ mg Ni/kg bw/day} * 0.1}{10 * 10 * 1 * 0.03 \text{ l/kg bw/day}} = 73 \text{ μg Ni/l}$$

The uncertainty factor UF_I accounting for interspecies variability is set to 10, assuming that humans are more sensitive than animals. The UF_{II} accounting for intraspecies variability is set to 10, reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 1, as the OECD TG 416 two-generation study is well described and well performed and since the highest dose level (2.2 mg Ni/kg bw/day) did not induce any signs of toxicity in the parental animals of both generations.

The calculated quality criterion of 73 µg Ni/l in this child-specific scenario is greater than the proposed quality criterion of 20 µg Ni/l implicating that young children are also protected by the proposed quality criterion.

Nickel, inorganic and soluble salts

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to nickel, inorganic and soluble salts. This resulted in 2010 in the present report which includes estimation of a quality criterion in drinking water for the mentioned substances.

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