European Food Safety Authority

EFSA Journal 2015;13(2):4002

SCIENTIFIC OPINION

Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

EFSA received a request from the Hellenic Food Authority (EFET) for a scientific opinion on the risk to human health from the presence of nickel (Ni) in food, particularly in vegetables. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) decided to extend the risk assessment also to drinking water. The reproductive and developmental toxicity in experimental animals was selected as the critical effect for the assessment of chronic effects of Ni. A tolerable daily intake of 2.8 µg Ni/kg body weight (b.w.) per day was derived from a lower 95 % confidence limit for a benchmark dose at 10 % extra risk ($BMDL_{10}$) of 0.28 mg/kg b.w. for post-implantation fetal loss in rats. The current dietary exposure to Ni raises concern when considering the mean and 95th percentile chronic exposure levels for all different age groups. The systemic contact dermatitis (SCD) elicited in Ni-sensitive humans after oral exposure to Ni was selected as the critical effect suitable for the assessment of acute effects of Ni. A lowest $BMDL_{10}$ of 1.1 µg Ni/kg b.w. was derived for the incidence of SCD following oral exposure to Ni of human volunteers. The CONTAM Panel applied a margin of exposure (MOE) approach and considered an MOE of 10 to be indicative of a low health concern. The MOEs calculated considering the estimated mean and the 95th percentile acute exposure levels were considerably below 10 for all age groups. Overall, the CONTAM Panel concluded that, at the current levels of acute dietary exposure to Ni, there is a concern that Ni-sensitized individuals may develop eczematous flare-up skin reactions. The CONTAM Panel noted the need for mechanistic studies to assess the human relevance of the effects on reproduction and development observed in experimental animals and for additional studies on human absorption of nickel from food, for example in combination with duplicate diet studies.

KEY WORDS

nickel, chemistry, human dietary exposure, toxicity, risk assessment, benchmark dose, margin of exposure (MOE), tolerable daily intake (TDI)

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[1] On request from the Hellenic Food Authority, Question No EFSA-Q-2012-00378, adopted on 22 January 2015.

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[3] Acknowledgements: The Panel wishes to thank the members of the Working Group on chromium and nickel: Michael DiNovi, Eugenia Dogliotti, Alessandro Di Domenico, Lutz Edler, Thierry Guérin, Antonio Mutti, Ivonne Rietjens (until 2 May 2014), Hendrik van Loveren and Christiane Vleminckx for the preparatory work on this scientific opinion, and EFSA staff: Davide Arcella, Marco Binaglia, Gina Cioacata, José Angel Gómez Ruiz, Ruth Roldán Torres and Eniko Varga for the support provided to this scientific opinion. The Panel acknowledges all European competent institutions that provided occurrence data on nickel in food and drinking water, and supported the data collection for the Comprehensive European Food Consumption Database.

Suggested citation: EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015. Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water. EFSA Journal 2015;13(2):4002, 202 pp. doi:10.2903/j.efsa.2015.4002

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

In March 2012, the European Food Safety Authority (EFSA) received a request from the Hellenic Food Authority (EFET) for a scientific opinion on the risk to human health for the presence of nickel (Ni) in food addressing particularly the presence of Ni in vegetables. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) decided to extend the risk assessment to Ni in water intended for human consumption and natural mineral waters, to assess their contribution to the dietary exposure to nickel.

Ni is a widespread component of Earth's surface. Its presence in food and drinking water is determined by both natural and anthropogenic factors, the latter generically identifiable with industrial and technological sources. In food and drinking water Ni generally occurs in the divalent form – Ni^{2+} or Ni(II) – its most stable oxidation state.

There are no maximum levels (MLs) for Ni in food. For drinking water, a parametric value of 20 µg Ni/L in water intended for human consumption, and a ML of 20 µg Ni/L in natural mineral waters are laid down in Council Directive 98/83/EC and in Commission Directive 2003/40/EC, respectively. These maximum limits are well within the guideline value of 70 µg/L set by the World Health Organization (WHO, 2005).

Following a call for data on Ni levels in food and drinking water (water intended for human consumption and mineral waters), a total of 18 885 food samples and 25 700 drinking water samples were available in the final dataset to estimate dietary exposure to nickel. No speciation data were provided. Samples were collected between 2003 and 2012 in 15 different European countries, with almost 80 % of the total collected in one Member State. The most reported analytical methods were inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS), that represented 54 % and 42 % of the methods reported, respectively. The highest sensitivity was reported for the analysis of drinking water with a limit of quantification (LOQ) of 0.001 µg/L (for both ICP-MS and AAS). In food, ICP-MS showed the lowest LOQ for the analysis of 'Alcoholic beverages' (0.002 µg/kg) while the lowest LOQ reported with AAS was 1 µg/kg for samples of 'Fish and seafood' and 'Sugar and confectionery'. In the final dataset, left-censored data represented 66 % of the analytical results, with 35 % in food samples and 89 % in drinking water samples.

At FoodEx level 1, all food groups were well represented, with a maximum of 25 700 samples of 'Drinking water' and 4 291 and 3 738 samples in the food groups 'Grain and grain-based products' and 'Vegetables and vegetable products (including fungi)', respectively. High mean levels of Ni were reported for 'Legumes, nuts and oilseeds' (~ 2 mg/kg), certain types of chocolate (cocoa) products (3.8 mg/kg), and 'Cocoa beans and cocoa products' (9.5 mg/kg).

The potential leaching of Ni into food from food contact material is not covered by the occurrence dataset used to estimate dietary exposure.

Chronic dietary exposure to Ni was estimated combining food mean occurrence data with food consumption data at the individual level. Mean chronic dietary exposure to nickel, across the different dietary surveys and age classes, ranged from 2.0 (minimum lower bound (LB), 'Elderly') to 13.1 µg/kg body weight (b.w.) per day (maximum upper bound (UB), 'Toddlers'). The 95th percentile dietary exposure ranged from 3.6 (minimum LB, 'Elderly') to 20.1 µg/kg b.w. per day (maximum UB, 'Toddlers'). Among the different age classes, 'Toddlers' and 'Other children' showed the highest chronic dietary exposure to nickel. Overall, the main contributors to the dietary exposure to nickel across the different dietary surveys and age classes were 'Grain and grain-based products', 'Non-alcoholic beverages (except milk-based beverages)', 'Sugar and confectionery', 'Legumes, nuts and oilseeds', and 'Vegetables and vegetable products (including fungi)'. 'Milk and dairy products' were also important contributors to the dietary exposure to nickel in the young population, in particular in toddlers. In the age classes 'Other children' and 'Adolescents' the relatively high consumption of chocolate and chocolate-based products made 'Sugar and confectionery' one of the main contributors.

The important role of 'Non-alcoholic beverages (except milk-based beverages)' in the dietary exposure to nickel is explained by the consumption of cocoa beverages and coffee in the young and adult population, respectively.

The contribution of 'Drinking water' to the total exposure to nickel was very small across dietary surveys and age classes (0.0005 %–1.7 %, LB-UB).

Highest levels for acute dietary exposure were observed in 'Toddlers' and 'Other children'. Mean dietary acute exposure in the young population ('Infants', 'Toddlers', 'Other children' and 'Adolescents') ranged from 3.4 (95 % confidence interval (CI) = 3.1–3.7) µg/kg b.w. in one survey for 'Adolescents' to 14.3 (95 % CI = 13.2–15.5) µg/kg b.w. in one survey for 'Toddlers'. The 95th percentile ranged from 8.6 (95 % CI = 8.0–9.1) µg/kg b.w. in one survey for 'Adolescents' to 35.0 (95 % CI = 26.8–47.2) µg/kg b.w. in one survey for 'Toddlers'. Mean dietary acute exposure in the adult population ('Adults', 'Elderly' and 'Very elderly') ranged from 2.5 (95 % CI = 2.2–2.9) µg/kg b.w. in one survey for 'Elderly' to 4.9 (95 % CI = 4.6–5.5) µg/kg b.w. in one survey for 'Adults'. The 95th percentile ranged from 5.5 (95 % CI = 5.1–6.0) µg/kg b.w. in one survey for 'Elderly' to 11.8 (95 % CI = 10.6–13.8) µg/kg b.w. in one survey for 'Adults'.

The CONTAM Panel concluded that the exposure via the diet likely represents the most important contribution to the overall exposure to Ni in the general population. Both for smokers and non-smokers not occupationally exposed to Ni, exposure by inhalation may be expected in general to represent a negligible or minor addition to the daily exposure via the diet.

Ni and Ni compounds have been classified by IARC (2012) as human carcinogens causing cancers of the lung, nasal cavity and paranasal sinuses after inhalation. There is currently no consistency in the epidemiological data to suggest that nickel compounds cause cancer at additional sites or by additional routes. Moreover, no tumours have been found in the oral carcinogenicity studies in experimental animals. Therefore, the CONTAM Panel considered it unlikely that dietary exposure to Ni results in cancer in humans.

In humans, non-carcinogenic health effects of oral exposure to Ni include effects on the gastrointestinal, haematological, neurological and immune system. Gastrointestinal and neurological symptoms were the most reported effects after acute exposure. Exposure through skin or by inhalation may lead to Ni sensitization. Whereas oral exposure to Ni is not known to lead to sensitization, oral absorption of Ni is able to elicit eczematous flare-up reactions in the skin in Ni-sensitized individuals.

In experimental animals, oral ingestion of soluble Ni salts has resulted in a wide range of adverse effects including nephrotoxicity/hepatotoxicity and metabolic effects. Ni is able to cross the placental barrier and exerts its primary toxic effects in experimental animals by affecting directly the developing embryo or fetus. Pre- and perinatal mortality were reported to be increased in the offspring of female rats ingesting Ni salts. These adverse effects occur at the lowest doses. Therefore, the CONTAM Panel identified reproductive and developmental toxicity as the critical effect for the risk characterization of chronic oral exposure to Ni. Benchmark dose (BMD) modelling was performed on a dose range finding 1-generation study, on a subsequent full 2-generation (2-GEN) study and on the combination of the data from the two studies. The CONTAM Panel noted that the use of combined data from the dose range finding and 2-GEN studies provided the most robust results and decided to use the results from this dataset for the selection of the reference point (RP). The Panel derived a tolerable daily intake (TDI) of 2.8 µg Ni/kg b.w. from a lower 95 % confidence limit for a benchmark dose at 10 % extra risk ($BMDL_{10}$) of 0.28 mg Ni /kg b.w. as calculated from the dose response analysis of the incidence of litters with post-implantation loss in rats, applying the default uncertainty factor of 100 to account for interspecies differences and human variability.

The mean chronic dietary exposure to Ni, across the different dietary surveys and age classes, ranging from 2.0 (minimum LB, 'Elderly') to 13.1 µg Ni/kg b.w. per day (maximum UB, 'Toddlers') is close to the TDI or above it particularly when considering the young age groups ('Infants', 'Other children',

'Toddlers' and 'Adolescents'). The 95th percentile dietary exposure ranging from 3.6 (minimum LB, 'Elderly') to 20.1 µg Ni/kg b.w. per day (maximum UB, 'Toddlers') is above the TDI for all age groups. Therefore, the CONTAM Panel concluded that the current chronic dietary exposure to Ni is of concern for the general population.

Although based on limited consumption data, the dietary exposure to Ni of the vegetarian population seems to be slightly higher than that estimated for the general population, with a highest estimated 95th percentile exposure of 7.1 µg Ni/kg b.w. per day. Therefore, the level of concern for dietary exposure to Ni for the general population can be extended to the vegetarian population.

It has been reported that individuals sensitised to nickel through dermal contact and who have allergic contact dermatitis (estimated prevalence in the general population to be up to 15 %, but frequently remaining undiagnosed) may develop eczematous flare-up reactions in the skin (systemic contact dermatitis, SCD) from oral exposure to nickel salts. The TDI of 2.8 µg Ni/kg b.w. per day may therefore not be sufficiently protective of individuals sensitized to nickel. Three studies analysing SCD elicited in Ni-sensitive humans after acute oral exposure to Ni were identified as suitable for dose-response analysis using the BMD approach. The Panel selected a lowest $BMDL_{10}$ of 1.1 µg Ni/kg b.w. from the dose-response analysis of these studies as an acute RP and adopted a margin of exposure (MOE) approach for risk characterization.

This selected RP is calculated on data obtained in a highly sensitive study group of fasted individuals given Ni sulphate in lactose capsules. Under these conditions, absorption is assumed to be considerably higher than from food. These considerations suggest that the selected RP could be conservative for the characterisation of the acute risks. On the other hand, the CONTAM Panel took into account the large inter-individual variability in the immune response that might not be covered by the limited number of individuals examined in the selected studies, and therefore decided that an MOE of 10 or higher would be indicative of a low health concern.

The MOEs calculated considering the estimated mean and the 95th percentile acute exposure levels were considerably below 10 for all age groups. Due to the approach followed for the derivation of the acute RP, it cannot be predicted whether all sensitized individuals will actually develop adverse reactions, nor what percentage eventually will develop such reactions at the estimated levels of Ni intake.

Overall, the CONTAM Panel concluded that, at the current levels of acute dietary exposure to Ni, there is a concern that Ni-sensitized individuals may develop eczematous flare-up skin reactions. The CONTAM Panel noted the need for mechanistic studies to assess the human relevance of the effects on reproduction and development observed in experimental animals and for additional studies on human absorption of Ni from food, for example in combination with duplicate diet studies.

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BACKGROUND AS PROVIDED BY THE HELLENIC FOOD AUTHORITY (EFET)

Nickel in its pure form is a hard, silvery-white metal that together with its compounds occurs naturally in the earth's crust. Discharge of nickel into the environment takes place as a result of both natural and anthropogenic activities (e.g. volcanic eruptions, windblown dust, forest fires, mining, smelting, manufacturing, combustion of fossil fuel, waste incineration etc.). Nickel is resistant to corrosion and heat, strength and hardness and it is often used in alloys and most commonly in stainless steel due to its physico-chemical properties. Nickel is widely distributed in nature and is present in water, soil, plants and animals.

Nickel compounds are classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) (IARC, 2012)[4] while metallic nickel and nickel alloys were classified as possibly carcinogenic to humans (Group 2B) (IARC, 1990).[5] Nickel has not been shown to be essential for humans.[6]

Exposure to nickel for the general non-smoking population is primarily from food and to a lesser extent via drinking water. Exposure to nickel through inhalation of ambient air is considered to be only a minor contributor to the overall exposure.

Presently there is no EU regulation regarding maximum levels of nickel in food. For drinking water, a quality standard of 20 µg/L for nickel is laid down in Council Directive 98/83/EC.[7] The WHO established a Tolerable Daily Intake (TDI) of 11 µg nickel/kg b.w. (WHO, 2007)[8] and from that derived a guideline value for drinking water of 70 µg nickel/L. The EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies concluded that it was not possible to establish a tolerable upper intake level for intake of nickel from food.[7]

TERMS OF REFERENCE AS PROVIDED BY THE HELLENIC FOOD AUTHORITY (EFET)

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the Hellenic Food Authority asks the European Food Safety Authority to provide a scientific opinion on the risk to human health related to the presence of nickel in food addressing particularly the presence of nickel in vegetables.

The scientific opinion should:

- Consider any relevant information on toxicity of nickel, considering all relevant toxicological endpoints;
- Assess the contribution of different foodstuffs to human exposure to nickel. This should particularly include the contribution of nickel in vegetables. An indication of non-dietary sources of exposure (e.g. air) should be given.
- Contain a dietary exposure assessment of nickel taking into account the recent analytical results on the occurrence on nickel in food, and the consumption patterns of specific (vulnerable) groups of the population (e.g. high consumers, children, people following a specific diet, etc.).
- Available biomonitoring data should be taken into account and the results be compared with the calculated exposure levels.

[4] IARC Monograph on the Evaluation of Carcinogenic Risks to Humans (2012). Nickel and Nickel compounds. Volume 100C, Available at: http://monographs.iarc.fr/ENG/Monographs/vol100C/mono100C-10.pdf

[5] IARC Monograph on the Evaluation of Carcinogenic Risks to Humans (1990). Chromium, Nickel and welding. Volume49, Available at: http://monographs.iarc.fr/ENG/Monographs/vol49/mono49.pdf

[6] Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Nickel. The EFSA Journal (2005) 146, 1-21. Available at http://www.efsa.europa.eu/en/efsajournal/doc/146.pdf

[7] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, OJ L 330 of 5.12.98, pp.32-54.

[8] WHO (2007). Nickel in Drinking Water – Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/07.08/55. Geneva: World Health Organization. Available at: http://www.who.int/water_sanitation_health/gdwqrevision/nickel2ndadd.pdf

ASSESSMENT

1. Introduction

Nickel (Ni) is a naturally occurring metal existing in various mineral forms and it is present in all compartments of the environment and ubiquitous in the biosphere. Ni is used in a wide variety of metallurgical processes such as electroplating and alloy production, and it is present in a wide range of consumer products. Ambient Ni concentrations reflect both natural and anthropogenic contributions. The anthropogenic emission rate is estimated to be higher (1.4–1.8 times) than the natural emission rate (IARC, 2012). Ni can exist in various oxidation states but the divalent form (Ni^{2+} or Ni(II)) – its most stable oxidation state – generally occurs in food and drinking water.

Ni is an essential micronutrient for higher plants and some animal species but there are no data proving that it is essential for humans. As for most metals, the toxicity of Ni is dependent on the route of exposure and the solubility of the Ni compound. The respiratory tract and the skin are the major routes of exposure for Ni-induced toxicity in the occupationally exposed population. Ni compounds are carcinogenic to humans after inhalation causing cancers of the lung, nasal cavity and paranasal sinuses (IARC, 2012). Allergic contact dermatitis is the most prevalent effect of Ni in the general population.

Dietary exposure and exposure via drinking water provide most of the intake of Ni. Ni absorption from the gastrointestinal tract in humans can vary significantly (between 1 and 40 %) depending on its chemical form, diet composition and fasting status. Ni is able to cross the placenta and oral exposure to soluble Ni compounds is associated with toxic effects in the developing embryo or fetus of experimental animals No tumours were found in animals that received soluble Ni compounds by the oral route. Consumption of Ni-rich food may elicit eczematous flare-up reactions in the skin in sensitized individuals (Systemic Ni Contact Dermatitis, SCD).

Presently there is no EU regulation regarding maximum levels of Ni in food. For drinking water, a parametric value of 20 µg Ni/L in water intended for human consumption and a Maximum Limit of 20 µg Ni/L in natural mineral waters are laid down in Council Directive 98/83/EC and in Commission Directive 2003/40/EC, respectively.

In March 2012, the European Food Safety Authority (EFSA) received a mandate from the Hellenic Food Authority (EFET) for a scientific opinion on estimation of the risk to human health from the presence of Ni in food, and total Cr in food and Cr(VI) in bottled water. The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) accepted the mandate and decided to deliver two separate Scientific Opinions. The CONTAM Panel also decided to extend the assessment to the presence of the two metals in water intended for human consumption, since in both cases it was considered to represent a significant contribution to the dietary exposure. The first Scientific Opinion on the risks to human health related to the presence of Cr in food and Cr(VI) in drinking water has been recently published (EFSA CONTAM Panel, 2014). Here, a scientific opinion on the risks for public health related to the presence of Ni in food and drinking water is presented.

1.1. Chemistry and physico-chemical properties

Nickel (Ni) was first isolated and recognised as a chemical element in 1751 by Axel F. Cronstedt. However, as the native metal, although rare, is almost always found alloyed with iron – e.g. the meteoric iron-nickel (Fe-Ni) alloy – its unintentional use can likely be traced back to the onset of iron metallurgy (approximately some 1300 BC in Europe). Ni elemental state, compounds, and minerals have been studied extensively, also due their important and wide range of industrial applications. In this Section, a short summary of the current knowledge on a number of physico-chemical properties and uses of the metal and its compounds is given.

The chemistry of Ni and Ni compounds is described in many general scientific references (e.g. WHO/IPCS, 1991; WHO, 2000, 2005; US EPA, 1986; Health Canada, 1994; Baralkiewicz and Siepak, 1999; Cotton et al., 1999; ATSDR, 2005; Kirk-Othmer, 2007; TCEQ, 2011). Due to the large number of scientific publications, technical reports and reviews, and educational and press releases available on these topics, no references are provided in the text unless specifically required.

1.1.1. General aspects

Ni (CAS registry No. 7440-02-0) is a widespread component of Earth's crust (approximately, 0.008 %); higher levels of the metal are likely present in Earth's core (8.5 %), deep-sea nodules (1.5 %), and meteorites (up to 50 %). Ni in agricultural soils has been reported at concentrations in the range from 3 to 1 000 mg/kg whereas its natural background levels in different water systems are generally below 2 µg/L. Most of the extremely rare, native Ni (Ni^0) on Earth comes from Fe-Ni meteorites, preserved from deterioration by the vacuum of space and fallen to earth not long ago; otherwise, native Ni, always in combination with iron, has been identified in very few geographic areas.
Aside from iron, Ni is also found in combination with other metals such as, for example, cobalt (Co), copper (Cu), and magnesium (Mg), and is extracted mostly from: sulphide ores containing pentlandite ($(Fe,Ni)_9S_8$), millerite (NiS), or certain varieties of pyrrhotite ($FenS_{n+1}$) with up to 3–5 % Ni; laterites or silicate/oxide ores containing garnierite (a Ni-rich serpentine with approximate composition $(Mg,Ni)_3Si_4O_{10}(OH)_3{\cdot}H_2O$) or nickeliferous limonite ($(Fe,Ni)O(OH){\cdot}nH_2O$); arsenide ores consisting of smaltite ($(Co,Fe,Ni)As_2$) or nickeline (NiAs). Millerite and nickeline, although rich in Ni, are rather limited sources of the metal. For the last part of the 19th century, Ni was mostly obtained from garnierite mines in New Caledonia. However, early in the 20th century Canada became the world's largest source of the metal following the exploitation of copper-nickel sulphide ores in Ontario. Australia, Canada, Indonesia, Philippines, and Russia have been the major primary Ni producers during the last few years, with more than 0.2 M tonnes/country per year (USGS, 2013); the worldwide production was estimated *ca*. 2.1 M tonnes in 2012. Ni sulphide ores are mostly mined underground using drilling, blasting, and other conventional techniques, whereas laterite Ni deposits are mined from surface pits using earth-moving equipment.

The route to extract Ni from ore is quite similar to that to obtain copper. Both sulphide ore concentrates and laterite ores are subjected to pyrometallurgical processes which basically involve three main sequential operations – i.e. roasting, smelting, and converting – and are similar for sulphide and laterite ores (in the latter case sulphur has to be added). Several Ni species are likely to be present during pyrometallurgical processing. In particular, two important substances are formed: Ni subsulphide (Ni_3S_2) when sulphur is abundant, and an iron-nickel alloy (containing up to 20–50 % Ni) when laterite ores are processed and no sulphur is added. Intermediate Ni products can undergo different types of refining steps. Pure Ni (99.9 %) can be produced by electrolytic refining. During vapometallurgical refining, impure metal obtained by reduction of Ni oxide is subjected to the action of carbon monoxide, a process yielding volatile Ni carbonyl ($Ni(CO)_4$) (Mond et al., 1890): heat can bring about $Ni(CO)_4$ decomposition into carbon monoxide and elemental Ni in the purest attainable form (≥ 99.97 %).

1.1.2. Uses and applications

Ni is primarily an alloy metal, and its main utilization (61 %) is in the many varieties of Ni steels and Ni cast irons (Bradley, 2011). The metal is used (26 %) in many industrial and consumer products such as AlNiCo magnets (typically 8–12 % Al, 15–26 % Ni, 5–24 % Co, ≤ 6 % Cu, ≤ 1 % Ti, and Fe to 100 %), coinage, rechargeable batteries, electric guitar strings, microphone capsules, and special alloys. Coinage may have a very high Ni content, close to 100 %. Ni foam or mesh is used for electrodes in alkaline fuel cells, while the metal itself or its alloys are frequently used as catalysts for hydrogenation reactions. Ni is also employed for electroplating (13 %) and other uses, namely in pigments and colours for ceramics and glassware, for Ni brasses and bronzes, in marine anti-fouling agents, and for alloys with aluminum, cobalt, chromium, copper, gold, lead, silver, and titanium. In particular, Ni may be present in white gold and in inexpensive alloys for fashion or junk jewellery

(including piercing); as to the latter, a Ni flash may be used in the silver or gold plating process of the base metal to provide a suitable surface for the silver or gold plating to adhere to (Bocca et al., 2007). Ni plating to strengthen metal against corrosion and wear as well as to improve its appearance was developed in the 1800s – quite before the development of commercial chrome plating – and has been used widely since the second half of the 19th century.

1.1.3. Physico-chemical properties

Ni is a silvery-white, hard, ductile metal and one of only few elemental metals which are magnetic at room temperature; bulk Ni is non-magnetic above approximately 350 °C (Curie point). The element has the basic physico-chemical properties described in Table 1.

Table 1: Some relevant physico-chemical properties of elemental nickel

Atomic number: 28	Boiling point: 2 730 °C (3 003 K)
Atomic mass: 58.69 amu	Vapour pressure: ≈ 1 Pa at 1 728 K; 100 kPa at 3 186 K
Chemical family: transition metals, d-block Group 10 (VIII-B) of periodic table	Density: 8.908 g/cm3 (at room temperature)
Electron configuration: [Ar] 4s 2 3d 8 or [Ar] 4s 1 3d 9	Solubility in water: practically insoluble
Electronegativity (Pauling scale): 1.91	Corrosion-resistant at room temperature. Reactive in air in powdered form, may spontaneously ignite
Melting point: 1 455 °C (1 728 K)	Dissolves readily in dilute mineral acids and *aqua regia* (nitro-hydrochloric acid) but is passivated by concentrated nitric acid. Highly resistant to attack by strong alkalis

Ni can exist in oxidation states –1, 0, +1, +2, +3, and +4. However, the divalent oxidation state (Ni^{2+} or Ni(II)) is the only one relevant under normal conditions: this is the oxidation state of importance in Ni aqueous and non-aqueous chemistry, with the exception, as to the latter, of a few particular complexes in other oxidation states. In natural waters (pH range of 5–9) not containing strong complexing agents, aqueous Ni(II) occurs mostly as the hexaquonickel ion $[Ni(H_2O)_6]^{2+}$; complexes with common ligands – HCO_3^-, Cl^-, OH^-, NH_3, SO_4^{2-}, etc. – are formed to a minor degree. Ni is slightly more resistant to oxidation than iron and cobalt: its standard potential at 25 °C is - 0.257 ± 0.008 V ($Ni^{2+} + 2e^- \rightarrow Ni^0$) (Bard et al., 1985). Several Ni compounds are commercially and environmentally relevant: some properties of a selection of these compounds are outlined in Table 2 and the text below.

On addition of CN^- ions to aqueous Ni(II), a green $Ni(CN)_2$ tetrahydrate precipitate is obtained; the solid hydrate can be converted to the yellow-brown anhydrous form by heating. With excess CN^- ions, complex ions are formed, such as $[Ni(CN)_4]^{2-}$ and $[Ni(CN)_5]^{3-}$, whose salts can also be crystallized. Similarly, various Ni(II) thiocyanate (SCN^-) derivatives are known.

Anhydrous Ni(II) halides are formed by direct reaction of the elements. Halides are soluble in water; from their aqueous solutions they can be crystallized as hydrates.

When aqueous solutions of Ni(II) salts are added with alkali metal hydroxides a green gel is obtained, that turns to a crystalline precipitate with time. $Ni(OH)_2$ is readily attacked by acids and has substantially no amphoteric properties.

Ni oxide is a green solid that can be formed by heating several Ni(II) compounds (e.g. carbonate, hydroxide, nitrate). It is insoluble in water but very reactive with acids. NiO is also the end product of heating Ni(III) sesquioxide ($Ni_2O_3 \cdot 2H_2O$), a black solid obtained by treating $Ni(OH)_2$ with oxidizing agents in an alkali metal hydroxide solution.

Several Ni(II) oxy acid salts are known, which are generally available as hydrates soluble in water; exceptions are, for instance, the $NiCO_3$ and $Ni_3(PO_4)_2$ hydrates.

Black NiS precipitates when sulphide ions are added to a solution containing Ni(II). With the exception of NiS, Ni sulphides are in general non-stoichiometric, their structures exhibiting Ni-Ni and Ni-S bonded interactions with features between ionic and covalent (Gibbs et al., 2005). The subsulphide Ni_3S_2 – formed during pyrometallurgical processing when sulphur is abundant but also composing the mineral heazlewoodite which sometimes is found in Ni ores – is the most Ni-rich sulphide species. Compared with other Ni sulphides, heazlewoodite is characterized by a greater preponderance of Ni-Ni metal bonded interactions and a greater level of covalency. The relatively high metallic conductivity of heazlewoodite can be related to the presence of four well-developed Ni-Ni bond paths (for the meaning of 'bond path' see Bader, 2009) that radiate from each Ni atom and form a contiguous array of Ni-Ni bond paths occurring throughout the entire structure, thereby creating an ideal circuit for electron transport. Heazlewoodite dissolution in neutral or acidic mediums was observed to release Ni(II) ions according to the following simplified reactions (Aromaa, 2011):

- $Ni_3S_2 \rightarrow 3\ Ni_2^{+} + 2\ S^0 + 6e^-$
- $Ni_3S_2 + 8\ H_2O \rightarrow 3\ Ni_2^{+} + 2\ SO_4^{2-} + 16H^{+} + 18\ e^-$

Ni(III) and Ni(IV) ions seldom occur in certain oxide systems and complexes that are relatively stable while, aside from Ni(CO)4, Ni^0 and Ni(I) compounds are even more infrequent. The higher oxidation states of Ni are characterized by strong oxidative potentials and are not stable in water (US EPA, 1986; IARC, 2012). However, the formation of Ni(II)-Ni(III) redox couple in cells is one of the proposed mechanisms for the generation of free active species that can induce oxidative processes *in vivo*, including oxidative DNA damage (Sunderman, 1989; Torreilles and Guérin, 1990; Chakrabarti et al., 2001; Chen et al., 2003a; Kasprzak et al., 2003).

Table 2: Some relevant nickel compounds and their basic properties. All data cross-checked with diverse literature and Internet sources.

Compound	Formula	CAS registry number	MW (amu)	Water solubility[a]	MP[b] (°C)	BP (°C)	Appearance
			Nickel^0 compounds				
Tetracarbonyl	$Ni(CO)_4$	13463-39-3	170.74	■	–19.3	3	Colourless, volatile liquid
			Nickel(II) compounds				
Acetate	$Ni(CH_3COO)_2$	373-02-4	176.78	■■	S[c]	–	Green powder (hydrate)
Ammonium sulphate	$Ni(NH_4)_2(SO_4)_2$	15699-18-0	286.90	■■	85–89	–[d]	Blue-green crystals
Bromide	$NiBr_2$	13462-88-9	218.50	■■■	963[e]	–[d]	Yellow-brown crystals
Carbonate	$NiCO_3$	3333-67-3	118.70	■	S[c]	–	Light-green powder
Chloride	$NiCl_2$	7718-54-9	129.60	■■■	1 001[e]	–[e]	Yellow-brown crystals
Cyanide	$Ni(CN)_2$	557-19-7	110.73	■	>200	–[c]	Yellow-brown salt
Hydroxide	$Ni(OH)_2$	12054-48-7	92.71	■	S[f]	–	Green powder (hydrate)
Nitrate	$Ni(NO_3)_2$	13138-45-9	182.70	■■■[g]	56.7[h]	136.7[h]	Green crystals (hydrate)
Oxide	NiO	1313-99-1	74.69	■	1955	–[d]	Green powder[i]
Phosphate	$Ni_3(PO_4)_2$	14396-43-1	366.02	■	S[d]	–[d]	Light-green powder (hydrate)
Subsulphide	Ni_3S_2	12035-72-2	240.21	■[h]	787	–[d]	Green[j]
Sulphamate	$Ni(NH_2SO_3)_2 \cdot 4H_2O$	124594-15-6	322.93	■■■[k]	S[d]	–[d]	Blue-green powder, crystals, or chunks
Sulphate	$NiSO_4$	7786-81-4	154.76	■■	840[c]	–	Greenish-yellow salt

BP: boiling point; CAS: Chemical Abstract Service; MW: molecular weight; MP: melting point.
(a): Generally reported at, or near room temperature: ■, slightly soluble or insoluble; ■■, fairly soluble; ■■■, very or freely soluble.
(b): 'S' (solid) indicates the physical state at or near room temperature, and is reported when a melting point is not available.
(c): Decomposes.
(d): No data available.
(e): Sublimes at or near melting point.
(f): Decomposes above 200 °C.
(g): Hexahydrate.
(h): From Ishimatsu et al. (1995).
(i): Different or non-stoichiometric compositions may be at the origin of black NiO forms (e.g. Ni_3O_4, Ni_2O_3).
(j): The green form of Ni_3S_2, stable at room temperature, turns to bronze-yellow at high temperature.
(k): From Maksin and Standritchuk (2007).

As is typical of transition metals, Ni compounds are characterized by an ample co-ordination chemistry, whose principal features may be summarized as follows: an octahedral geometry is associated with coordination number 6 (Figure 1) and with the oxidation states Ni(II), Ni(III), and Ni(IV); besides, Ni^0 and Ni(II) exhibit a tetrahedral geometry with coordination number 4 (Figure 2); the latter also characterizes the square planar geometry that can be found in some Ni compounds (Figure 3). Much of Ni chemistry deals with Lewis acid-base coordination complexes, in which ligands (ions or molecules) bind to the coordinating metal (atom or ion): ligands act as electron-pair donors (Lewis bases) while the metal acts as an electron-pair acceptor (Lewis acid) owing to its valence-shell orbitals that can accommodate electron pairs. Therefore, ligands must have at least one pair of electrons suitable for being donated to the metal. The metal-ligand bonding can have various degrees of covalent nature even when both Ni and ligands are formally ionic species. Many paramagnetic Ni complexes have an octahedral geometry. Ni forms complexes (chelates) that are insoluble in water, but soluble in organic solvents: these compounds are often very stable and can play a role in trace analysis.

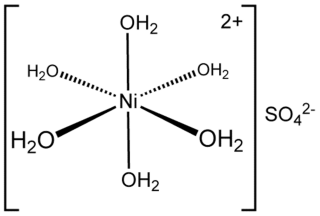


Figure 1: Example of octahedral geometry: Ni(II) ion solvate

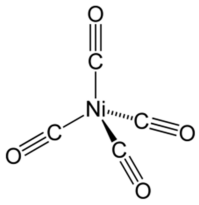


Figure 2: Example of tetrahedral geometry: Ni^0 tetracarbonyl

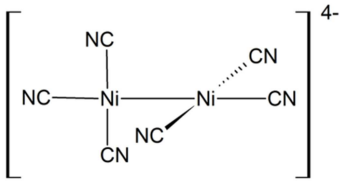


Figure 3: Example of square planar geometry: hexacyanodi-nickelate(I) ion

1.1.4. Natural and artificial isotopes

There are five naturally-occurring stable Ni isotopes, with mass numbers 58 (68.07 %), 60 (26.23 %), 61 (1.14 %), 62 (3.63 %), and 64 (0.93 %). Several radioactive isotopes are also known: with the exception of ^{59}Ni and ^{63}Ni, whose half-lives are 76 000 and 100 years, respectively, they all exhibit short half-lives, in the order of a few days or, in general, much shorter. With the exception of ^{59}Ni, of cosmic origin, all the other radioactive isotopes have an artificial origin. ^{59}Ni has found applications in isotope geology; ^{63}Ni, whose decay is by 0.067-MeV β^- emission only, has several technical uses, including instrumental analytical chemistry (electron capture detectors for gas chromatographs). There are no uses of Ni radionuclides in medical/biological research.

1.2. Conclusion

Ni is a widespread component of the Earth's surface: it is generally found as the divalent ion Ni^{2+} (Ni(II)) in different minerals, in combination with cobalt, copper, iron, and/or magnesium; native Ni (Ni^0) in combination with native iron (Fe^0) can rarely be encountered. In aqueous media Ni generally occurs in the form of its most stable oxidation state, Ni(II). Ni is widely used in the production of many varieties of iron-Ni alloys, in countless industrial and consumer products, in electroplating, in pigments and colours for ceramics and glassware, in marine anti-fouling agents, and in alloys with aluminum, cobalt, chromium, copper, gold, lead, silver, and titanium. Ni may be present in white gold and in inexpensive alloys for fashion or junk jewellery (including piercing); a Ni flash may also be used in the silver or gold plating process of the aforesaid jewellery.

1.3. Environmental fate and sources of food and drinking water contamination

1.3.1. Enviromental fate

Ni is a naturally occurring element due to a variety of processes, it is present in all compartments of the environment and ubiquitous in the biosphere. However, ambient Ni concentrations may also reflect anthropogenic contributions that can add up detectably to background concentrations arising from natural sources and processes such as bedrock weathering and erosion. Environmental exposure to Ni of anthropogenic origin occurs locally from, among others: emissions of metal mining, smelting, and refining operations; industrial activities (Ni plating, alloy manufacturing, etc.); land disposal of sludges, solids, and slags; disposal of effluents. Diffuse sources may arise from combustion of fossil fuels, waste incineration, wood combustion, etc. (WHO/IPCS, 1991). In general, a wide variability characterizes ambient Ni concentrations, reflecting the influence of Ni emissions from different types of sources.

In the atmosphere Ni occurs mostly as fine respirable particles – $Ø_a$ (aerodynamic diameter) < 2 µm – eventually suspended onto particulate matter (NRCC, 1981; US EPA, 1986). Anthropogenic sources of air-borne Ni account for more than 80 % of the atmospheric Ni burden, whereas the remainder to 100 % is accounted for by natural sources such as soil dust, volcanoes, forest fires, etc. (WHO/IPCS, 1991; Chau and Kulikovsky-Cordeiro, 1995; Eisler, 1998). In the early 1980s, worldwide atmospheric emissions of Ni by natural and anthropogenic sources were estimated respectively at around 26 and 43 k tonnes/year, 0.9 k tonnes/year being produced from gasoline and diesel fuel combustion (WHO/IPCS, 1991; EU RAR, 2008).

Ni enters ambient waters primarily as Ni-containing particulate matter carried by rainwater and through the degradation/dissolution of primary bedrock materials and soils (US EPA, 1986; WHO/IPCS, 1991). The main anthropogenic sources of Ni in water are primary Ni production, metallurgical processes, combustion and incineration of fossil fuels, chemical and catalyst production, and discharges of industrial and municipal wastes. In aquatic systems, Ni soluble salts are in general carried by clay particles, organic matter, and other substances; in surface and ground waters at natural pH values, Ni occurs mostly as hydrated Ni(II) ions although it can also form strong soluble complexes with OH^-, HCO_3^-, and SO_4^{2-}. Under anaerobic conditions (e.g. in deep ground waters), Ni can be segregated from the environment as insoluble sulphide. The medium acidity, redox potential, ionic strength, ligands' type and concentration, and adsorption on solid surfaces are determinants of Ni fate in fresh and marine waters (US EPA, 1980; WHO/IPCS, 1991; Chau and Kulikovsky-Cordeiro, 1995).

Ni is released to soils from smelting and refining operations, disposal of sewage sludge, or use of sludge as a fertilizer; secondary anthropogenic sources include emissions from motor vehicles and electric power utilities (US EPA, 1986). Weathering and erosion of geological materials are natural sources of Ni to soils (Chau and Kulikovsky-Cordeiro, 1995).

Concentrations of Ni in unpolluted atmospheres and in pristine surface waters may be so low as to be near the limits of current analytical methods (ATSDR, 2005).

1.3.2. Sources of food and drinking water contamination

1.3.2.1. Nickel in air

Over the European territory, atmospheric Ni concentrations in remote areas that are considered to be substantially free of anthropogenic Ni emissions are in the range of 1–3 ng/m^3 (WHO/IPCS, 1991). In 1996, in Finnish Lapland, Ni background concentration was approximately 0.51 ng/m^3, whereas in south-west Finland background Ni levels in air were found to vary between 2 and 8 ng/m^3 (EU RAR, 2008). Ni levels measured in the late 1990s in several European cities of various countries – i.e. Denmark, France, Greece, and the United Kingdom – fell in the range 1–20 ng/m^3 (annual mean estimates) (EU RAR, 2008). In Germany, the annual mean Ni concentrations in air at eight different

locations were quite low, in that they were comprised between 0.61 and 1.70 ng/m^3. The highest Ni values in air were measured in the Catalunya region (Spain), the average Ni concentrations being comprised between 9 and 74 ng/m^3.

Over the period 1985–1996, Ni concentrations in rainfall in Sweden were seen to vary in the range 0.1–0.8 µg/L, in natural areas levels being between 0.11 and 0.35 µg/L (EU RAR, 2008). Dissolved Ni in wet deposition sampled in central Greece varied from 2 to 18 µg/L.

Ni concentrations in air detected in several Canadian cities over the 1987–1990 period fell in the range 1–20 ng/m^3 (annual mean estimates); at sites near copper and zinc metallurgical plants, mean concentrations were between 5 and 151 ng/m^3; at remote and rural Canadian sites, mean concentrations could reach approximately 1 ng/m^3, but on the whole were substantially lower (Health Canada, 1994; Newhook et al., 2003; ATSDR, 2005). Relative to the rest of the year, Ni levels exhibited higher values during winter, a possible indication of the role of combustion sources in the environmental release of Ni.

In the US environment, Ni concentrations in air-borne particulate were found to be in the range 0.01–60, 0.6–78, and 1–328 ng/m^3 in remote, rural, and urban areas, respectively (Schroeder et al., 1987), although typical mean values fell between 6 and 20 ng/m^3 (Barceloux, 1999). Ni levels in air-borne dust were seen to vary remarkably: for instance, values as high as 150 ng/m^3 were detected near anthropogenic sources of the metal (Barceloux, 1999), whereas measurements carried out on air dust ($Ø_a < 10$ µm) collected in the area of Spokane (Washington), from January 1995 to March 1999, yielded a mean 1.2 ng/m^3 (Claiborn et al., 2002). According to US EPA (2001), the average Ni concentration in the air of the contiguous States was 2.22 ng/m^3; the five States with the highest average concentrations were characterized by Ni values between 3.69 and 6.60 ng/m^3, whereas the five States with the lowest levels had average values in the range 0.127–0.311 ng/m^3. When compared with older determinations available from monitoring ambient air of many US cities since the 1960s, data suggest that atmospheric Ni concentrations in the US are characterized by a downward trend (US EPA, 1986). The two major contributing sources to Ni presence in urban air were suggested to be oil and coal combustion (ATSDR, 2005). In other cases, the Ni levels measured in air particulate matter – similar results for $Ø_a \leq 10$ µm and $Ø_a \leq 2.5$ µm – at certain urban sites were attributed to emissions from zinc smelters and steel mills/oil combustion (Sweet et al., 1993).

The exposure to Ni of high school students in New York City was extensively investigated in the winter and summer of 1999 (Kinney et al., 2002). Mean Ni concentrations in air particulate ($Ø_a \leq 2.5$ µm) sampled outdoor, indoor, and with personal monitors in the winter were 32.3, 31.6, and 49.6 ng/m^3, respectively: these values are characterized by large dispersions and may be viewed as similar. The corresponding mean Ni concentrations obtained during the summer survey were found to be 11.7, 12.6, and 17.3 ng/m^3, somewhat lower than the winter concentrations but again characterized by relatively large dispersions. Different indoor concentrations were found in other investigations of US urban environments (Chicago area, counties of New York State, Maryland): in all cases Ni values were on average in the order of 1–3 ng/m^3 and in general < 10 ng/m^3 (Koutrakis et al., 1992; van Winkle and Scheff, 2001; Graney et al., 2004).

1.3.2.2. Nickel in water bodies

Ni concentrations in sea water fall generally between 0.1 and 3 µg/L (Barceloux, 1999). In marine open waters of the European region (including the Celtic Sea, the English Channel, the Irish Sea, the Portuguese and Spanish coastal waters, and the North Sea), the typical average concentrations of dissolved Ni were reported to range 0.14–0.33 µg/L (Burton et al., 1993; Tappin et al., 1995; Hall et al., 1996; Statham et al., 1999; Cotté-Krief et al., 2000, 2002). Typical concentrations of the metal measured in the open Atlantic Ocean were in the same range. Water samples collected in the Atlantic Ocean – from the surface, 400 m depth, and the Atlantic shelf – were found to have Ni levels of 0.106, 0.158, and 0.205 µg/L, respectively. Ni concentrations in surface marine waters were found to be sensitive to salinity and phosphorus concentration (van Geen et al., 1988; Yeats, 1988). Higher levels

of dissolved Ni (0.64–0.81 µg/L) were measured in different areas of the Baltic Sea (Kremling et al., 1997; Kremling and Streu, 2000), while estuaries and estuary-influenced coastal waters were reported to exhibit even higher contamination levels (EU RAR, 2008). In the South San Francisco bay Ni concentrations were detected at about 3 µg/L, with a large fraction of the metal complexed by strong organic ligands (Donat et al., 1994).

Uncontaminated fresh waters typically contain about 0.3 µg/L of Ni (Barceloux, 1999). Ni's natural background concentrations can rarely be found in most European aquatic and terrestrial compartments as a consequence of its prolonged anthropogenic inputs from diffuse sources. The background values described hereafter are related to virtually pristine waters. Ni concentrations measured in surficial samples from different fresh water bodies in Finland, Lapland, and northern Sweden were comprised in the range < 0.1–1 µg/L, with average values between 0.11 and 0.54 µg/L (Borg, 1987; Verta et al., 1990; Mannio et al., 1995). Contrary to these low values, Ni concentrations in natural streams in western Finland were reported to be quite higher, the highest levels being detected in a stream draining an area covered for more than 80 % with fine sulphidic silts and sediments (EU RAR, 2008). Background Ni concentrations of 9.0 and 1.3 µg/L were respectively measured in the Ivel and Yare rivers in the United Kingdom (Bubb and Lester, 1996). Zuurdeeg et al. (1992) – as reported by EU RAR (2008) – developed a worldwide database containing many entries of surface waters selected for cleanliness: average global background concentration estimates for dissolved and total Ni were 0.25 µg/L (0.064 –0.99 µg/L) and 3.03 µg/L (1.88–4.89 µg/L), respectively.

Ni concentrations in the lower Mississippi river ranged from 1.2 to 1.5 µg/L in samples taken at different flow conditions (Shiller and Boyle, 1987). In an extensive 1977–1979 study of ground and surface waters throughout New Jersey, the median Ni levels in either type of water samples were both 3.0 µg/L (ATSDR, 2005); the respective 90th percentiles were 11 and 10 µg/L. However, Ni levels as high as 600 µg/L were reported for ground water but not characterized as to the source of contamination, although local Ni-plating activities may be responsible for high levels of Ni in ground water (IARC, 1990). Ni concentrations were measured in several ground water samples from an alluvial aquifer underlying Denver (Colorado) (Bruce and McMahon, 1996): the range of values was 1–20 µg/L with a median of 3 µg/L. Samples represented an environment exposed to commercial, industrial, residential, and agricultural activities. Ni was determined in streams and creeks under the impact of abandoned or active mining operations in South Dakota (May et al., 2001). In these surface waters, Ni concentrations were generally in the range 1.3–7.6 µg/L, reaching occasionally higher values (up to 20 µg/L): concentrations were highest near the merging with drainage waters from the mining area. Median and maximum Ni concentrations in the Lake Huron in 1980 were estimated as 0.54 and 3.8 µg/L (ATSDR, 2005). In 1982, Ni concentrations in the lake Ontario (Hamilton Harbor) ranged < 1–17 µg/L, with a median of 6 µg/L (Poulton, 1987). In a 1993 survey of heavy metal concentrations in the Great Lakes, average Ni concentrations of 0.872 and 0.752 µg/L were measured in lakes Erie and Ontario, respectively (Nriagu et al., 1996). Concentrations were highest in near-shore waters due to the proximity to urban sites and polluted river mouths.

The outcome of a 1969–1970 survey of 969 water supplies in the US representing eight metropolitan areas and one State was (fraction of samples, Ni concentration): 21.7 %, < 1 µg/L; 43.2 %, 1–5 µg/L; 25.6 %, 6–10 µg/L; 8.5 %, 11–20 µg/L; 1 %, > 20 µg/L (ATSDR, 2005).

Ni was detected in rainwater collected in different locations and times. In samples from US (Delaware and New Hampshire) and Canada (Ontario) the annual mean Ni concentrations exhibited a limited variability (0.56–0.79 µg/L) (Chan et al., 1986; Barrie et al., 1987; Feng et al., 2000). Rainwater samples collected over the period 1985–1990 in remote regions of the Atlantic Ocean were found to have Ni levels between 0.63 and 1.42 µg/L (Helmers and Schrems, 1995). In cloud water sampled in 1993 in Washington State, Ni was present on average at 0.5 µg/L (0.2 ng/m^3) (Vong et al., 1997). Snow from Montreal (Canada) exhibited Ni concentrations in the range 2–300 µg/L while the particulate matter present in snow had a Ni content of 100–500 µg/L (ATSDR, 2005): Ni contamination was suggested to arise from oil combustion.

1.3.2.3. Nickel in sediments and soil

Frink (1996) reported (geometric) mean Ni soil concentrations comprised in the range 4.4–61 mg/kg d.w. (dry weight) for 12 different European countries/areas, nine of which appear to be also present in the 15-country group discussed below: based on the 12 means available, a median Ni concentration of 21.7 mg/kg d.w. was estimated for European soils. From the assay of topsoil samples collected in 15 European Union (EU) countries, and in fair agreement with the aforementioned data, Ni concentrations were found to range < 2–2 560 mg/kg d.w., with a median of 14 mg/kg d.w. (EU RAR, 2008). This value may be considered as a typical background concentration of the metal in the European region although, especially for larger countries, background levels can show a remarkable variability mainly caused by geological factors. For the soil compartment, typical average background concentrations of 2.7 mg/kg d.w. (Denmark) to more than 64 mg/kg d.w. (Greece) were found.

The Ni content in soil can vary remarkably depending on local geology (for instance, ultramafic rocks are rich in Ni) (ATSDR, 2005). A Ni content of 0.5 % (5 000 mg/kg) is common in south-eastern US, while high Ni concentrations are not unusual in glacial till (south-eastern Canada). In Australia, north of Sydney, Ni concentrations as high as 2 030 mg/kg d.w. were reported in topsoils naturally enriched in Ni through the weathering of underlying haematite, magnetite, quartz, and kaolinite minerals (Lottermoser, 2002). A topsoil survey throughout the US reported that Ni concentrations ranged from < 5 to 700 mg/kg d.w., with a geometric mean of 13 mg/kg d.w., whereas cultivated soils exhibited Ni levels between 5 and 500 mg/kg d.w. and a typical concentration of 50 mg/kg d.w. (ATSDR, 2005). Mean Ni concentrations in the forest floors of nine north-eastern States were on average 11 mg/kg (Friedland et al., 1986).

Ni concentrations in contaminated soils within approximately eight km of a Ni smelter in the Sudbury region (Ontario) ranged from 80 to 5 100 mg/kg d.w. Ni concentrations appeared to decline with increasing distance from the smelter, with a logarithmic trend that could be explained with Ni accumulations resulting from atmospheric deposition and soil runoff (ATSDR, 2005). In a later soil survey in the same region, soil samples were taken in relation to the three local smelters Copper Cliff, Coniston, and Falconbridge: Ni concentration ranges (and means) were of 80–2 149 (580), 156–628 (286), and 23–475 (210) mg/kg d.w. (Adamo et al., 1996). In an agricultural area of Ontario (Canada), Ni levels ranged from 4.0 to 48 mg/kg d.w. (mean, 16.2 mg/kg d.w.) in numerous untreated soils, whereas the mean concentration in soils treated with sludges was significantly higher (20 mg/kg d.w.) in spite of a similar contamination range (6.2–34 mg/kg d.w.) (Webber and Shamess, 1987).

Only few background concentrations for Ni in European fresh water sediments, presumably pristine, are available from the literature: average values for samples from Belgium, Germany, Luxembourg, and the Netherlands are comprised between 9 and 36 mg/kg d.w. (Crommentuijn et al., 1997; EU RAR, 2008; Swennen et al., 1998). Mean Ni levels in pristine sediment from sites off the northern coast of Alaska ranged from 25 to 31 mg/kg (Sweeney and Naidu, 1989): Ni was mostly associated with silt and clay. Background concentrations of the metal in sediment samples from lake St. Clair fell in the range 8.5–21.1 mg/kg (Rossmann, 1988). The average Ni concentrations in surface sediment from several other US water bodies (lakes and river basins of the Rocky Mountains area) ranged from 6.4 to 38 mg/kg d.w. (Maret and Skinner, 2000; ATSDR, 2005). Based on an extensive investigation on 541 streambed-sediment samples from throughout the conterminous US (Rice, 1999), a median Ni concentration of 27 mg/kg d.w. was obtained (range: 6–530 mg/kg d.w.): Ni appeared to be highly associated with fine-grained sediment with a higher organic carbon content. On the whole, sediment is an important sink for Ni in water, so that Ni content in sediments is expected to be high near sources of Ni emissions (May et al., 2001).

1.3.2.4. Nickel release into food during preparation

In general, Ni-containing food contact materials are made of highly corrosion-resistant stainless steel so that the metal should not migrate into food in quantities that would endanger human health (EDQM, 2013). Stainless steel products are used in food transportation (e.g. milk tankers), for food processing equipment and containers, for brew kettles and beer kegs, for cooking utensils and

tableware, in slaughter-houses, for electric kettles, for different kinds of kitchen appliances, etc. Ni release from Ni-plated kitchenware may also be a source of dietary exposure to Ni: Ni plating is not as resistant to corrosion as stainless steel and for this reason Ni-plated articles are not normally used for materials that are meant to be in contact with food.

A pilot study was carried out by Cubadda et al. (2003) to examine the effect of technological processing on the content of Ni (and other elements) in the production of pasta. The effect of cooking was also investigated. Cereal milling was observed to reduce the Ni content by an average 65 % (dry weight basis): on the whole, commercial pasta exhibited low average levels of the element. Cooking also determined a significant Ni decrease in the pasta samples tested by approximately 50 % (dry weight basis).

Stainless steel pots of different origins were tested by boiling 350 mL of 5 % acetic acid for 5 minutes (Kuligowski and Halperin, 1992). The resulting Ni concentrations in the acid medium were found to be in the range 0.01–0.21 mg/L, whereas substantially no Ni ($\leq$ 0.03 mg/L) was released from the assayed kitchen utensils made of cast iron, mild steel, aluminum, or porcelain-enamelled steel: it was observed that the better the stainless steel quality, the less the corrosion and derived Ni release. Flint and Packirisamy (1995) showed that, except for the atypically high releases detected when new pans were first used, the contribution made by stainless steel cookware to Ni in the diet was very small and within the normal daily variation of Ni intake. The use of new stainless steel pans to cook acidic fruits determined an increase of Ni levels that, in the worst case observed, was estimated to be in the order of one-fifth the average daily dietary exposure to the metal reported at that time (approximately 0.2 mg/person). Accominotti et al. (1998) compared Ni levels in habitual menus cooked with glass and stainless steel cookwares: the differences observed did not appear to be relevant in relation to the ensuing dietary exposure, and their conclusion was that there is no advantage for Ni-sensitive persons in avoiding the use of stainless steel cookware if it is of good quality.

According to the outcome of a study carried out by Berg et al. (2000) on models sampled from the Danish market in 1994, electric kettles with stainless steel heating elements - or with gold- or Teflon-coated elements - did not release Ni to drinking water in relevant quantities: the maximum level observed was in the order of 0.030 mg/L but in most cases no release was detected (< 0.001 mg/L). On the contrary, electric kettles and immersion heaters with open Ni- or chromium-plated heating coils were seen to transfer Ni in amounts of up to approximately 0.5 mg/L, especially after descaling. As had previously been reported by Flint and Packirisamy (1995), also Berg et al. (2000) observed a decrease of Ni release to water with use. Bolle et al. (2011) detected a relatively high release of Ni into tea infusions - up to a few mg/L for a contact time of 30 minutes at boiling temperature - from brass teapots in which Ni was in general present at percent fraction level. Ni release increased even remarkably when plain tea was replaced with tea containing citric acid or with a citric acid solution (1 g/L). The aforesaid teapots, purchased in Brussels but made in Africa and India, were later withdrawn from market.

At present, as recommended by the Council of Europe, manufacturers of food preparation and handling tools and equipment made of stainless steel should respect the migration of Ni compliant with a specific release limit (SRL) of 0.14 mg/kg food (EDQM, 2013).

1.3.3. Conclusions

Ni occurs in environmental compartments and in the biosphere with highly variable levels, normally as Ni(II) compounds or complexes. The metal presence is determined by natural as well as anthropogenic factors, the latter generically identifiable with industrial and technological sources. A wide variability characterizes ambient Ni concentrations reflecting the influence of Ni emissions from different types of sources.

In air, Ni occurs mostly as fine respirable particles that are removed by wet and dry deposition. Anthropogenic sources of air-borne Ni account for more than 80 % of the atmospheric Ni burden; the

remainder to 100 % is accounted for by natural sources. In non-industrialized areas, background Ni concentrations are generally around or below 3 ng/m^3 (yearly averages), although higher levels have also been observed; in urban and industrialized areas Ni concentrations in air can be considerably higher (up to tens or hundreds of ng/m^3). In rainwater, Ni concentrations are on average measured in the range < 1 µg/L, although greater levels have been detected depending on location.

Surface runoff, deposition from air, and release of municipal and industrial waste waters are sources of Ni in surface waters. Under anaerobic conditions, typical of deep waters, Ni can be segregated from the environment as insoluble sulphide. Although in surface waters total Ni may be present at levels greater than a few µg/L, in general the element is detected at average concentrations in the order of 3 µg/L or lower, rivers being more contaminated than lakes and sea water. Total Ni concentrations in ground water and water from drinking water sources/supplies may range from less than 1 µg/L up to few tens of µg/L, although cases of a high Ni occurrence (up to hundreds of µg/L) have also been reported.

Ni is released to soils from smelting and refining operations, disposal of sewage sludge, or use of sludge as a fertilizer; secondary anthropogenic sources include emissions from motor vehicles and electric power utilities. Weathering and erosion of geological materials are natural sources of Ni to soils. Typical average background concentrations of Ni in topsoil are in the order of few tens of mg/kg (namely, < 50 mg/kg): these values are consistent with Ni levels that on a local basis can be even remarkably higher, and with concentration ranges of two or three orders of magnitude. Reflecting the extent of anthropogenic impact, Ni concentrations are on average higher in agricultural soils while reaching the highest values in soils proximal to industrial activities.

Sediments are an important sink for Ni in water. In general, Ni concentrations detected in such matrix show similarities with those detected in topsoil: in particular, Ni content in sediments is expected to be high near sources of Ni emissions.

Migration from food contact material could represent an additional source for the presence of Ni in food and drinking water. The CONTAM Panel concluded that the extent of Ni migration into food and drinking water due to the use of good quality stainless steel cookware, tableware, and in general food contact materials has likely little or no relevance compared to the dietary exposure determined by the intrinsic presence of Ni in diet constituents. However, leaching of Ni into food may not be negligible for food contact materials made of poor quality stainless steel, or of other metal alloys containing Ni.

1.4. Previous risk assessments

Several evaluations of the carcinogenicity of Ni and Ni compounds have been performed by the International Agency for Research on Cancer (IARC) working groups (IARC, 1973, 1976, 1979, 1982, 1987, 1990). The most recent is the monograph on Ni and Ni compounds (IARC, 2012). On the basis of new data available IARC concluded that 'There is sufficient evidence in humans for the carcinogenicity of mixtures that include Ni compounds and Ni metal. These agents cause cancers of the lung and of the nasal cavity and paranasal sinuses'. Ni compounds are classified as carcinogenic to humans (group 1).

In 2000 the World Health Organization (WHO) (WHO, 2000) reviewed the toxicological properties and health effects of Ni. WHO identified food and water as relevant sources of Ni intake (estimated daily Ni intake < 300 µg and < 20 µg, respectively) but concluded that the gastrointestinal uptake is of limited interest for effects other than Ni hypersensitivity. It was reported that Ni seems to be essential for humans, although no data are available concerning Ni deficiency.

The WHO (1993) established a tolerable daily intake (TDI) of 5 µg/kg body weight (b.w.) per day for Ni. This TDI was derived from a NOAEL of 5 mg/kg b.w. per day from a dietary 2-year study with rats exposed to Ni sulphate hexahydrate (Ambrose et al., 1976) in which altered organ-to-body weight ratios were observed, using an uncertainty factor of 1 000 (100 for inter- and intraspecies variation and an additional factor of 10 to compensate for the lack of adequate studies on long-term exposure and

reproductive effects, a lack of data on carcinogenicity by the oral route and a much higher intestinal absorption when taken on an empty stomach in drinking- water than when taken together with food). The provisional drinking water quality guideline of 20 µg/L was established by assuming a 60 kg adult drinks 2 litres of water and allocating 10 % of the TDI to drinking water. This guideline value was maintained in the addendum to the 2nd edition published in 1998.

In the background document on drinking-water quality in 2005, the WHO established a TDI of 22 µg/kg b.w. per day from a critical no-observed-adverse-effect level (NOAEL) of 2.2 mg Ni/kg b.w. per day for all the end-points studied in an oral (gavage) two-generation reproduction study of Ni sulphate hexahydrate on rats (SLI, 2000b) by applying an uncertainty factor of 100. This TDI is higher than the previous one but the SLI (2000b) study was considered a better reproductive study with less uncertainty as compared to the Ambrose et al. (1976) 2-generation (2-GEN) study. A 'general toxicity value' of 130 µg/L (rounded value) could be determined from this TDI by assuming a 60 kg adult drinks 2 litres of water per day and allocating a conservative 20 % of the TDI to drinking-water. It was noted that this value may not be sufficiently protective of individuals sensitized to Ni, for whom a sufficiently high oral challenge has been shown to elicit an eczematous flare-up reaction. The guideline value for Ni in drinking-water is therefore derived using the lowest-observed-adverse-effect level (LOAEL) of 12 µg/kg b.w. established after provocation of fasted patients with an empty stomach (Nielsen et al., 1999). This is considered by WHO 'a worst case scenario' since the absorption of Ni from drinking water on an empty stomach is 10 to 40 fold higher than the absorption from food. Because this LOAEL of 12 µg/kg b.w. is based on a highly sensitive human population, no additional uncertainty factor was included to derive the TDI. Assuming a 60 kg adult drinking 2 litres of water per day and allocating 20 % of total daily intake to drinking-water, WHO established the guideline value of 70 µg/litre (rounded value), which would be considered protecting Ni-sensitive individuals, the group at risk.

The Food Safety Committee of Japan (FSCJ, 2012) established a TDI of 4 µg/kg b.w. using the same study (Nielsen et al., 1999) used by WHO but applying an uncertainty factor of 3 (for using a LOAEL close to a NOAEL) to the LOAEL of 12 µg/kg b.w. The Japanese Committee on Drinking Water Quality therefore established a guideline value of 20 µg /L.

The US-Environmental Protection Agency (US EPA, 1996) derived an oral reference dose (RfD) of 20 µg/kg b.w. per day for water-soluble Ni salts based on decreased body and organ weights in a 2-year feeding study in rats (Ambrose et al., 1976) in which a NOAEL of 5 mg/kg b.w. per day was reported, and by applying a 300-fold UF. A subchronic study conducted by American Biogenics Corporation (1988) also indicated 5 mg/kg b.w. per day to be a NOAEL, which supported the Ambrose et al. (1976) chronic NOAEL of 5 mg/kg b.w. per day. In addition to the standard uncertainty factor of 100, an additional factor 3 was used to account for inadequacies in the reproductive studies. EPA concluded that there was medium confidence in this RfD, based on high mortality in the control group. Regarding the carcinogenic potential of oral exposure to soluble Ni according to EPA it 'cannot be determined because there are inadequate data to perform an assessment'.

The Health Canada (1994) has evaluated soluble Ni compounds and has derived a tolerable intake (TI) of 0.05 mg Ni/kg-day for Ni sulphate and a TI of 0.0013 mg Ni/kg b.w. per day for Ni chloride. The TI for Ni sulphate is based on the Ambrose et al. (1976) study by applying an UF of 100. The TI for Ni chloride is based on the reproductive toxicity study by Smith et al (1993). In this study female rats drank Ni chloride solutions for 11 weeks prior to mating and then during two successive gestation and lactation periods. Perinatal toxicity was observed with a LOAEL of 10 mg/L Ni (1.3 mg Ni/kg b.w. per day). An uncertainty factor of 1 000 was applied that included a factor of 10 for intraspecies variation, a factor of 10 for interspecies variation and a factor of 10 for the use of a LOAEL rather than a NOAEL.

The TERA (Toxicology Excellence for Risk Assessment) assessed the toxicity of Ni on behalf of the Metal Finishing Association of Southern California (Inc.), the US EPA, and Health Canada (TERA,

1999). In its assessment, TERA derived a RfD for soluble Ni salts of 8 µg/kg b.w. per day based on the lowest LOAEL from Vyskocil et al. (1994) study where multiple sensitive endpoints for kidney function were evaluated in rats exposed to soluble Ni compounds in drinking water for 6 months. Kidney weights were significantly increased in the exposed groups but no significant changes were observed in other parameters. This study only tested one dose and only tested 10 animals/sex/exposure duration and therefore the TERA considered the confidence in the supporting database medium and the overall confidence in the RfD low.

The National Institute for Public Health and the Environment (RIVM) (RIVM, 1991, 2001) established a TDI of 50 µg/kg b.w. per day for Ni, based on the NOAEL from the Ambrose et al. (1976) study, using an uncertainty factor of 100.

The Expert Group on Vitamins and Minerals (EVM) (EVM, 2003) concluded that the carcinogenicity of Ni compounds by inhalation in occupational settings does not appear to be relevant to oral exposure from low levels in foods, although data are lacking. The toxicity of Ni in animal studies indicates a decrease in b.w. in dogs and an increase in kidney weight at doses of 70 mg/kg b.w. per day. In reproductive toxicity studies in rats, although there were no effects on reproduction, there was an increase in the number of pups stillborn or dying shortly after birth with the numbers of stillborn increasing as a function of dose from 5 to 50 mg/kg b.w. per day. Moreover, there was a significant decrease in b.w. of the pups at 50 mg/kg b.w. per day. It was noted that the key toxic endpoint for Ni in humans is the aggravation of Ni sensitization which is possible at the levels of Ni found in food (levels as low as 0.49 to 0.72 mg/day may be able to trigger a reaction particularly if taken on an empty stomach).

The Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2005) reviewed the toxicological profile of Ni. The ATSDR noted that intermediate-duration studies suggest that the developing organism may be a sensitive target of Ni toxicity. However, due to inadequate studies, no acute- or intermediate-duration oral minimal risk level (MRL) was derived. Also the data on chronic toxicity were considered to be inadequate to derive a chronic MRL.

The EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies (EFSA, 2005) received a request from the Commission to provide a scientific opinion on the tolerable upper intake level of Ni. It was noted that there is no evidence that Ni is essential for humans. It was observed that perinatal mortality was reported to be increased in the offspring of female rats ingesting Ni salts, even at the lowest administered dose (1.3 mg Ni/kg b.w. per day). Since oral intakes of Ni as low as about 500 µg/day (about 8 µg/kg b.w. per day) have been reported to aggravate hand eczema in Ni sensitised subjects and in absence of adequate dose-response data for these effects, EFSA considered it not possible to establish a TDI.

The European Union Risk Assessment Report (EU RAR, 2008) reviewed the toxicological profile of Ni and Ni compounds. Separate human health risk assessments addressed each of the priority Ni compounds (Ni metal, Ni sulphate, Ni carbonate, Ni chloride and Ni dinitrate). The need for further studies to evaluate the possible genotoxic effects of metallic Ni was identified. In the case of Ni sulphate and Ni chloride it was noted that there was a need for further studies to evaluate the possible effects on germ cells. A comprehensive exposure assessment for all identified routes of exposure was performed in the EU RAR (2008) for the occupational exposure, the exposure to consumers and the indirect exposure via the environment. At the regional scale the dietary exposure estimates (based on a literature survey for Ni dietary intake in the EU) resulted as the most important pathway accounting for > 95 % of the total exposure. The estimated median external dietary exposure levels were 1.6 µg Ni/kg b.w. per day in adults (considering a b.w. of 70 kg) and 5.5 µg Ni/kg b.w. per day in 1–2 years old children (considering an average b.w. of 11.5 kg as reported in the EU RAR, 2008), and the 95th percentile levels were 3.4 and 9.3 µg Ni/kg b.w. per day for the two age classes, respectively. External exposure estimates via tap water were approximately two orders of magnitude lower than exposure levels via food. To allow for the risk assessment for systemic effects considering the total exposure to Ni, internal doses were estimated taking into account the specific absorption factors for

the different routes of exposure (e.g. 100 % for exposure by inhalation, 30 % for exposure via drinking water (assuming fasting conditions) and 5 % for exposure via food (assuming non-fasting conditions)). For the hazard assessment, the EU considered the LOAEL of 12 µg Ni/kg b.w. for the dermal elicitation in severely sensitised individuals (Nielsen et al., 1999) and a NOAEL of 1.1 mg Ni/kg b.w. per day for developmental effects observed in a 2-GEN study in rats (SLI, 2000b) as the key reference points (RPs) for the calculation of the Margins of Safety (MOS) for systemic effects in the general population. Similarly to what was done in the exposure assessment, internal doses were calculated for the two RPs, resulting in an absorbed LOAEL of 3.6 µg Ni/kg b.w. for sensitising effects and an absorbed NOAEL of 0.055 mg Ni/kg b.w. per day for the developmental effects, considering fasting and non-fasting conditions, respectively. For the sensitising effects, a MOS of 7 to the absorbed LOAEL of 3.6 µg Ni/kg b.w. was considered of concern for the severely sensitized people. MOS in the range of 200-300 to the NOAEL of 0.055 mg Ni/kg b.w. per day were considered for the effects on reproduction under a conservative approach.

The conclusion was reached that there was no concern for the general population that are not already sensitised to Ni from oral exposure to Ni metal, Ni sulphate and Ni chloride. In the case of Ni carbonate and Ni dinitrate it was concluded that there is no known consumer exposure. However, patients with severe Ni sensitisation constitute a particular sensitive population to oral challenge with Ni and are potentially at risk from excessive exposure to Ni in food and water. EU concluded that additional risk reduction measures may be needed to protect this sensitive population.

The Office of Environmental Health Hazard Assessment (OEHHA) in the review of its Scientific Review Panel on Ni reference exposure levels (RELs) (OEHHA, 2011) identified a chronic oral REL for Ni of 0.011 mg Ni/kg b.w per day from a NOAEL of 1.1 mg Ni/kg b.w. per day for developmental effects as derived from a 2-GEN study in rats (SLI, 2000b). The oral REL was estimated by using an uncertainty factor of 10 each for interspecies and intraspecies extrapolations.

The health-based guidance values (HBGVs) established in previous assessments are summarised in Table 3.

Table 3: Overview of the health-based guidance values established by institutional bodies for nickel

Organisation	Limit type	Health-based guidance value (µg/kg b.w. per day)	Species	Reference point (mg/kg b.w. per day)	Critical effect	Reference	UF
FSCJ (2012)	TDI	4	Fasted human	LOAEL 0.012	Eczematous reaction	Nielsen et al. (1990)	3
OEHHA (2011)	REL	11	Rat	NOAEL	Increased pup mortality	SLI (2000b)	10
WHO (2005)	TDI	12	Fasted human	LOAEL 0.012	Eczematous reaction	Nielsen et al. (1990)	1[(a)]
RIVM (2001)	TDI	50	Rat	NOAEL	Decreased organ and body weight	Ambrose et al. (1976)	100
TERA (1999)	RfD	8	Rat	LOAEL 8	Increased kidney weight	Vyskocil et al (1994)	1 000
Health Canada (1994)	TI Ni sulphate	50	Rat	NOAEL 5	Decreased organ and body weight	Ambrose et al. (1976)	100
	TI Ni chloride	1.3	Rat	LOAEL 1.3	Increased pup mortality	George et al. (1989) Smith et al. (1993)	1 000

Organisation	Limit type	Health-based guidance value (µg/kg b.w. per day)	Species	Reference point (mg/kg b.w. per day)	Critical effect	Reference	UF
US EPA (1996)	RfD	20 (soluble Ni salts)	Rat	NOAEL 5	Decreased organ and body weight	Ambrose et al. (1976)	300

b.w.: body weight; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level; REL: reference exposure level; RfD: reference dose; TI: tolerable intake; TDI: tolerable daily intake; UF: uncertainty factor.
(a): No uncertainty factor applied.

2. Legislation

EU Council Directive 98/83/EC[9] 'on the quality of water intended for human consumption' sets a parametric value for Ni at 20 µg/L (Annex I, Part B 'Chemical parameters'); at the same time, it also indicates the minimum performance characteristics to be warranted by the method used for the analysis (Annex III). Within the Directive scope, water intended for human consumption refers to:

- 'all water … intended for drinking, cooking, food preparation or other domestic purposes, … from a distribution network, from a tanker, or in bottles or containers;'
- 'all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption …'.

In the EU, the concentration limit for Ni in natural mineral waters is regulated by the Commission Directive 2003/40/EC.[10] In this Directive, Ni is listed in Annex I amongst the constituents naturally present in natural mineral waters, with a maximum limit of 20 µg/L. As above, the Directive also indicates the performance characteristics to be warranted by the method used for the analysis (Annex II).

The aforesaid maximum limits are well within the guideline value of 70 µg/L µg/L set by the World Health Organization (WHO, 2007).

No regulatory limits or quality standards are currently available in the USA (US EPA, 2015) and Canada (Health Canada, 2012) for Ni in drinking water. For US drinking water, a 100 µg/L maximum level was extant until early 1995, when the limit was remanded to be reconsidered (US EPA, 2015). According to Australian drinking water guidelines, Ni concentration should not exceed 20 µg/L (Australian Government, 2014).

There are currently no maximum levels in the EU legislation for Ni in food. There is also no regulatory limit for release of Ni from food contact materials in the EU. However, the Council of Europe recently published a practical guide on metals and alloys used in food contact materials and articles, which set out a specific release limit (SRL) for Ni of 0.14 mg/kg food (EDQM, 2013).

According to the REACH Regulation (EC) 1907/2006,[11] elemental Ni and several Ni derivatives are included in the list of substances subject to restrictions for their marketing (Annex XVII 'Restrictions on the manufacture, placing on the market, and use of certain dangerous substances, preparations, and

[9] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 1–28.
[10] Commission Directive 2003/40/EC of 16 May 2003 establishing the list, concentration limits, and labelling requirements for the constituents of natural mineral waters and the conditions for using ozone-enriched air for the treatment of natural mineral waters and spring waters. OJ L126 22.5.2003, p. 34–39.
[11] Regulation (EC) 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) 793/93 and Commission Regulation (EC) 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC, and 2000/21/EC.

articles'). In particular, restrictions including maximum rates of release are expressed for those consumer articles containing elemental Ni and intended to come into direct and prolonged contact with the skin (e.g. jewellery or garments).

3. Sampling and methods of analysis

3.1. Sample collection and storage

There are no specific guidelines for the sampling of foods to be analysed for their total Ni. Therefore, basic rules for sampling of trace elements should be followed. For example, requirements are laid down in Commission Regulation (EC) No 333/2007[12] amended by Commission Regulation (EU) No 836/2011[13] for methods of sampling and analysis for the official control of some trace elements in certain foodstuffs. This Regulation contains *inter alia* a number of provisions concerning methods of sampling depending on the size of the lot, packaging, transport, storage, sealing and labelling. The primary objective is to obtain a representative and homogeneous laboratory sample with no secondary contamination.

The EN 13804:2013 standard on the general consideration and specific requirements for the determination of elements and their chemical species does not address sampling issues but it details processes involved from receipt of the laboratory sample to the end result. Both laboratory samples and test samples shall be stored in such a way that the composition and sample mass does not change as a result of, for instance, drying out, evaporative loss, spoilage or decay.

Minimum frequency of sampling and analysis for water intended for human consumption is laid down in Council Directive 98/83/EC. For water, sampling, preservation and handling are described in different parts of EN ISO 5667 standard (EN ISO 5667-1:2007; EN ISO 5667-3:2012; EN ISO 5667-5:2006).

For Ni analysis in waters, samples are collected in acid cleaned polyethylene, polypropylene, perfluoro ethylene/propylene, polytetrafluoroethylene, polyethylene high density, or perfluoroalkoxy polymer containers and acidified to pH 1 to pH 2 with HNO_3 before storage. Perfluoroalkoxy polymers and perfluoro ethylene/propylen are recommended for low concentrations. Samples remain stable for a maximum of six months (EN ISO 5667-3:2012).

3.2. Methods of analysis

3.2.1. Food sample preparation

The analyst must ensure that samples do not become contaminated during sample preparation. Wherever possible, apparatus and equipments that come into contact with the sample should not contain Ni and should be made of inert materials, e.g. titanium or ceramic knives, agate mortar or ball mill for size reduction and homogenisation instead of stainless steel or iron equipment. These should be acid cleaned to minimise the risk of contamination (EN 13804:2013). Food samples are commonly treated in the same way as is done before consumption (washed, peeled, removal of non-edible parts). Examples of sample preparation procedures for some foodstuffs are given in EN 13804:2013.

[12] Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs OJ L 88, 29.3.2007, p.29–38.

[13] Commission Regulation (EU) No 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. OJ L 215, 20.8.2011, p. 9–16.

3.2.2. Instrumental techniques

3.2.2.1. Nickel analysis

The methods of analysis of Ni in water and food samples have been reviewed by ATSDR (2005). The most common methods used to detect Ni in food and water samples, with or without preconcentration or separation steps, are atomic absorption spectrometry (AAS), either flame or graphite furnace (FAAS, GFAAS), inductively coupled plasma optical/atomic emission or mass spectrometry (ICP-OES/ICP-AES or ICP-MS), followed by spectrophotometric techniques (ultra-violet (UV)-visible absorption, photodiode array) (ATSDR, 2005). In some studies, it was reported that Ni^{2+} ion was analysed (Liu et al., 2004; Rekha et al., 2007; Ghaedi et al., 2009; Vargas et al., 2009; Sykuła-Zajac et al., 2010; Tokalıoğlu and Daşdelen, 2011; Dobrowolski and Otto, 2012; Baran and Yasar, 2012; Bahadir et al., 2013; Khani and Shemirani, 2013). Finally, one recent study analysed Ni speciation (separation of Ni^{2+} ionic species, Ni–quinate and Ni–citrate complexes) in tea infusions by monolithic chromatography and ICPMS or Q-TOF-MS detection (Ščančar et al., 2013a).

In water samples, the limit of detection (LOD) ranged from 0.05 µg/L to 1.05 µg/L depending on the pre-concentration and the detection techniques used (Table 4). Analytical techniques with a LOD > 2 µg/L do not comply with Council Directive 98/83/EC and were not included in Table 4.

Table 4: LOD for nickel analysis in waters according to the analytical method used

Detection technique	Pre-concentration technique (Y/N)	LOD (µg/L)	Reference
UV–Visible	Y	0.12-0.17	Liu et al. (2004)
UV–Visible	Y	0.05	Rekha et al. (2007)
UV–Visible	Y	0.32	Khani and Shemirani (2013)
FAAS	Y	1.05	Citak et al. (2009)
FAAS	Y	0.43	Tokalıoğlu and Daşdelen (2011)
FAAS	Y	0.7	Bahadir et al. (2013)
FAAS	Y	(a)< 0.5	ISO 8288:1986
GFAAS	N	1	EN ISO 15586: 2004
ICP-OES	N	1	EN ISO 11885: 2009
ICP-MS	N	1	EN ISO 17294-2: 2003
ICP-MS	N	0.5	Millour et al. (2011)

FAAS: Flame atomic absorption spectrometry; GFAAS: Graphite furnace atomic absorption spectrometry; ICP-OES: Inductively coupled plasma optical emission spectrometry; ICP-MS: Inductively coupled plasma mass spectrometry; LOD: limit of detection; UV: ultraviolet.
(a): no LOD indicated, estimation based on optimal working range given for method C of ISO 8288:1986.

In foods, there is a wide variation of LODs ranging from 2 µg/kg by electro-thermal vaporisation inductively coupled plasma mass spectrometry (ETV-ICP-MS) to 290 µg/kg by FAAS, and between 0.006 µg/L by ICP-MS and 117 µg/L by FAAS (Table 5). Inductively coupled plasma - optical/atomic emission spectrometry (ICP-OES / ICP-AES) or mass spectrometry (ICP-MS) are increasingly being used, due to their multielement capacity and sensitivity (Chaves et al., 2010; Cindric et al., 2011; Millour et al., 2011; Altundag and Tuzen, 2011; Karadas and Kara, 2012; Ščančar et al., 2013a).

Table 5: LOD for nickel analysis in foods according to the analytical method used

Detection technique	Pre-concentration technique (Y/N)	LOD (µg/kg)	Type of food	Reference
UV–Visible	Y	240	Vegetables	Liu et al. (2004)
UV–Visible	Y	ni[(a)]	Teas	Sykuła-Zajac et al. (2010)
UV–Visible	Y	0.32[(b)]	Vegetables	Khani and Shemirani (2013)
AdSV	N	47[(b)]	Fish	Vargas et al. (2009)
FAAS	Y	1.1[(b)]	Vegetables, cereals, chocolate	Ferreira et al. (2001)

Table 5: LOD for nickel analysis in foods according to the analytical method used (continued)

Detection technique	Pre-concentration technique (Y/N)	LOD (µg/kg)	Type of food	Reference
FAAS	Y	2.1[(b)]	Fruits, vegetables	Ghaedi et al. (2009)
FAAS	Y	5.0[(b)]	Leaves	Silva et al. (2009)
FAAS	N	117[(b)]	Vegetables	dos Santos Salazar et al. (2011)
FAAS	Y	1.41[(b)]	Tea, spices, herbs	Soylak and Aydin (2011)
FAAS	Y	290	Edible oils	Baran and Yasar (2012)
FAAS	Y	0.6[(b)]	Vegetable	Zarei and Shemirani (2012)
FAAS	Y	0.98[(b)]	Fruits, vegetables	Behbahani et al. (2013)
FAAS	Y	3.2[(b)]	Alcoholic beverages	Ribeiro et al. (2013)
FAAS	N	42[(b)]	Red wines	Santos et al. (2013)
GFAAS	N	22	Vegetables	Gottelt et al. (1996)
GFAAS	N	< 250[(c)]	Animal and vegetable fats and oils	ISO 8294:1994
GFAAS	N	13	Vegetable oils, margarine, butter	Ieggli et al. (2011)
GFAAS	Y	20	Vegetables	Dobrowolski and Otto (2012)
GFAAS	Y	13	Fish	Imyim et al. (2013)
GFAAS	N	0.9[(b)]	Teas	Shaltout et al. (2013)
ICP-OES	N	ni	Dried fruits	Altundag and Tuzen (2011)
ICP-OES	N	79[(b)]	Apple juices	Cindric et al. (2011)
ICP-AES	N	ni	Herbal teas, teas infusion	Szymczycha-Madeja et al. (2013)
ICP-MS	Y	4.3	Cereals	Huang and Jiang (2010)
ETV-ICP-MS	Y	2	Vegetable seeds	Chaves et al. (2010)
ICP-MS	N	0.006[(b)]	Wines	Catarino et al. (2006)
ICP-MS	N	42	All foods & beverages	Millour et al. (2011)
ICP-MS	N	34	Honey	Chudzinska et al. (2012)
ICP-MS	N	ni	Spices, herbs	Karadas and Kara (2012)
ICP-MS	N	6[(b)]	Teas	Ščančar et al. (2013a)

AdsV: adsorptive stripping voltammetry; ETV: electrothermal vaporization; ETV-ICP-MS: electro-thermal vaporisation inductively coupled plasma mass spectrometry; FAAS: Flame atomic absorption spectrometry; GFAAS: Graphite furnace atomic absorption spectrometry; ICP-OES or AES: Inductively coupled plasma optical/atomic emission spectrometry; ICP-MS: Inductively coupled plasma mass spectrometry; LOD: limit of detection; N: no; ni: not indicated; UV: ultraviolet; Y: yes.
(a): no LOD indicated;
(b): given in µg/L;
(c): no LOD indicated, estimation based on optimal working range given.

3.2.3. Analytical quality assurance: performance criteria, reference materials, validation and proficiency testing

Some performance criteria (limits of detection and quantification (LOD/LOQ), method bias and recovery, measurement uncertainties and analytical quality assurance) for the determination of total Ni content in food are laid down in the EN 13804:2013 standard. The LOD and LOQ will vary with the analytical technique, the sample mass, the laboratory and the food matrix.

For the determination of Ni in water intended for human consumption, Council Directive 98/83/EC indicates that the performance characteristics for the method of analysis used must, as a minimum, be capable of measuring concentrations equal to the parametric value with a trueness, precision and limit of detection that must not exceed 10 % of the parametric value (i.e. LOD ≤ 2 µg/L).

To demonstrate the trueness (i.e. systematic error) and precision (i.e. random error) of trace element data, one of the important criteria is the reporting of correct (and precise) data for the Ni content of certified reference materials that closely match the matrix of the samples under investigation (Jorhem,

2004). Several standard or certified reference materials (SRMs and CRMs) are available for total Ni (Appendix A, Table A1).

Four standardised methods are available for the determination of total Ni in water by FAAS after chelation and extraction ISO 8288:1986) or graphite furnace atomic absorption spectrometry (GFAAS; EN ISO 15586: 2004), by inductively coupled plasma optical emission spectrometry (ICP-OES) (EN ISO 11885:2009) or mass spectrometry (ICP-MS) (EN ISO 17294-1:2004 and EN ISO 17294-2:2003). Similar sensitivity can be obtained by these methods (LOD of 1 µg/L by GFAAS, ICP-OES and ICP-MS or < 0.5 µg/L by FAAS after chelation and extraction). No standardised method is available for the determination of total Ni in food but a standard exists for animal and vegetable fats and oils by graphite furnace atomic absorption spectrometry (GFAAS) after pressure digestion with a LOD of < 250 µg/kg (ISO 8294:1994).

A number of proficiency testing schemes (PTs) are regularly organised by several providers[14] for measurement of Ni in food and in water to demonstrate and maintain analytical quality assurance. For example, Food Analysis Performance Assessment Scheme (FAPAS) organized several proficiency tests on the determination of total Ni in potable water (e.g. LEAP® Scheme reports CHEM107, 109, 111V2 and 112 in 2012 and 2013) and 77–94 % of 17 to 26 participants obtained satisfactory results at the level of interest (range: 10.3–20.9 µg/L). The Bureau Interprofessionnel d'Etudes Analytiques (Bipea) organised four rounds per annual series on trace elements (including Ni) in plants (on average 35 participants from nine different countries), in sea products (on average 33 participants from eight different countries), in food (on average 33 participants from six different countries) and six rounds per annual series on physicochemical analyses (including Ni) in feed and surface water (on average 95 participants from 13 different countries). Between 2010 and 2013, 93 to 99 % of the results for total Ni in feed water (assigned values ranging from 13 to 95 µg/L; 59 to 67 participants) were considered satisfactory by Bipea (Bipea reports n° 2010–2011–0415 or -525; n° 2011–2012–0448 and - 557; n° 2012–2013–0123).

3.3. Conclusions

In summary, several analytical techniques are suitable for the determination of total Ni in foods and waters. F- or GF-AAS, and increasingly ICP-OES or ICP-MS have been used. In water samples, LOD ranged from 0.05 µg/L to 1.05 µg/L depending on the pre-concentration and the detection techniques used (Table 4). In foods, there is a wide variation of LODs ranging from 2 µg/kg by ETV-ICP-MS to 290 µg/kg by FAAS and from 0.006 µg/L by ICP-MS to 117 µg/L by FAAS (Table 5).

One European standardised method for the determination of total Ni only in animal and vegetable fats and oils by GFAAS (LOD of < 250 µg/kg) is available (ISO 8294:1994) while four standardised methods are available in water by F- or GF-AAS or ICP-(OES or MS) techniques with LOD of 1 µg/L by GFAAS, ICP-OES and ICP-MS or < 0.5 µg/L by FAAS after chelation and extraction (ISO 8288:1986; EN ISO 17294-1:2004 and EN ISO 17294-2:2003; EN ISO 15586:2004; EN ISO 11885:2009).

To demonstrate and maintain analytical quality assurance, several SRMs and CRMs are available and regular proficiency testing schemes are organised for total Ni in food and water.

4. Occurrence of nickel in food and drinking water

4.1. Previously reported occurrence results

4.1.1. Nickel in food

There is a very large number of data in the literature as regards occurrence data of Ni in food. All the analytical results are reported on a wet weight basis unless specified as dry weight (d.w.) or lack of

[14] http://www.eptis.bam.de/php/eptis/index.php?task=show__search_pt_scheme

information. In general, food was reported to contain Ni at concentrations less than 0.5 mg/kg (IARC, 1990, 2012; WHO, 2000, 2007; Leblanc et al., 2005; Cempel and Nikel, 2006; FSA, 2006; Duda-Chodak and Blaszczyk, 2008; Rose et al., 2010; Becker and Kumpulainen, 2011; Noël et al., 2012). The highest mean concentrations of Ni have been measured in wild growing edible mushrooms, cocoa or cocoa-based products (containing > 10 mg/kg dry weight), beans, seeds, nuts and grains (e.g. cocoa beans, 9.8 mg/kg; soybeans, 5.2 mg/kg; soya products, 5.1 mg/kg; walnuts, 3.6 mg/kg; peanuts, 2.8 mg/kg; oats, 2.3 mg/kg; buckwheat, 2.0 mg/kg; and oatmeal, 1.8 mg/kg) (IARC, 1990, 2012; EVM, 2002; ATSDR, 2005; WHO, 2007; Duda-Chodak and Blaszczyk, 2008; Kalač, 2010; OEHHA, 2011; Ščančar et al., 2013b). Ni concentrations of about 30 µg/L and of 100 µg/L have been found in beer and wine; respectively (IARC, 1990). Factors influencing the concentration of Ni in food include the type of food (e.g. grains, vegetables, fruits versus seafood, mother's milk versus cow's milk), growing conditions (i.e. higher concentrations have been observed in food grown in areas of high environmental or soil contamination), and food preparation techniques (e.g. Ni content of cooking utensils, although the evidence for leaching from stainless steel cookware is somewhat mixed (IARC, 2012).

The Ni content determined in 11 pooled samples of the most consumed baby foods in Europe during the first nine months of life (including infant formulae and solid foods and beverages), sampled from six different countries (France, Germany, Italy, Portugal, Sweden and the UK) and in baby foods from the 'national baskets' of four selected countries (Italy, Spain, Slovakia, and Sweden) ranged from 0.1 to 1.3 mg/kg (Pandelova et al., 2012).

In Croatia, the Ni concentrations determined in 72 milk samples ranged from 0.072 to 0.097 mg/L (Vahčić et al., 2010).

In France, in the first TDS (n = 998), the food groups containing most Ni on average were nuts and oilseed, chocolate and breakfast cereals at respective average levels of 1.15, 0.63 and 0.55 mg/kg; other food groups contain less than 0.5 mg/kg (Leblanc et al., 2005). Of the 1 319 food samples analysed for the second total diet study (TDS), the highest mean levels were found in the food group 'sweeteners, honey and confectionery' (0.798 mg/kg) followed by 'ice cream' (0.353 mg/kg), 'tofu' (0.351 mg/kg; n = 2) and 'cereals and cereal products' (0.155 mg/kg) (Noël et al., 2012). For the remaining food groups, concentrations ranged from 0.057 mg/kg (fat and oils) to 0.137 mg/kg (cooked dishes and snacks). In a specific study of fish and other seafood from the French market (n = 159), Ni was found at an average level of 0.074 mg/kg in fish and 0.299 mg/kg in seafood (Guérin et al., 2011). Amongst fish, tuna, pilchard and pout had the highest levels of Ni (0.341, 0.236 and 0.161 mg/kg, respectively) and amongst seafood, cockle contained the highest level (2.8 mg/kg) followed by periwinkle (0.709 mg/kg).

The Ni levels in food duplicates (7-day sampling period) consumed by 42 young German children (four to seven years old) were in the range of 0.069–2.0 mg/kg d.w. (geometric mean 0.348 mg/kg d.w.) (Wittsiepe et al., 2009).

Ni contents in yolk and albumen eggs of domestic avian species (chicken, turkey, duck, goose, and pigeon; n = 120) deriving from 24 birds of each species, reared in the same poultry farm in northern Greece varied between 0.012 and 0.074 mg/kg (Nisianakis et al., 2009). The Ni content in ten wild edible mushroom species from West Macedonia and Epirus, regions of Northern Greece, ranged from 0.76 to 9.93 mg/kg d.w. (Ouzouni et al., 2009). In carrots, onions, and potatoes (n = 30) cultivated in the industrial zone of Asopos region, central Greece, Ni concentrations were found up to nine times higher than those cultivated in other Greek areas (controls) (Kirkillis et al., 2012). Averages of 0.80, 0.474 or 0.422 mg/kg were found in potatoes, carrots and onions, respectively instead of 0.078, 0.093 or 0.057 mg/kg in control samples.

In nine fresh and dried samples of Hungarian apricots, the highest Ni content were 0.425 and 2.14 mg/kg, respectively (range 0.116–2.14 mg/kg) (Davarynejad et al., 2012).

Average Ni concentrations in101 samples of eight different Italian rice grain collected in four regions ranged from 0.15 to 0.48 mg/kg d.w. (Sommella et al., 2013). The Ni concentrations found in 15 fish and seven cephalopod molluscs, caught in the southern Adriatic Sea, were higher in cephalopods (mean 2.12 mg/kg) than fish (mean 1.13 mg/kg); however, relatively high concentrations were found in pink cuttlefish (3.97 mg/kg), elegant cuttlefish (2.77 mg/kg), Mediterranean horse mackerel (2.72 mg/kg), megrim (2.31 mg/kg) and horse mackerel (1.82 mg/kg) (Storelli, 2009). Another Italian study indicated that Ni ranges in the meat of 148 wild and bred animals were 0.2–6.7 mg/kg d.w. (mean 1.4 mg/kg d.w.; n = 70) for farm animals and 0.4 to 5.7 mg/kg d.w. (mean 1.7 mg/kg d.w.; n = 78) for large game (Desideri et al., 2012).

In Poland, Ni content ranged from 0.16 to 26.5 mg/kg d.w. in 30 samples of herbs and spices (Bielicka-Gieldon and Rylko, 2013), from 0.11 to 1.76 mg/kg in 10 samples of margarines (Lodyga-Chruscinska et al., 2012), from 0.023 to 1.33 mg/kg in 30 samples of honey (Madejczyk and Baralkiewicz, 2008), from 0.42 to 1.83 mg/kg (mean 0.90 mg/kg) in six samples of honey (Nowak et al., 2011), from 0.82 to 7.88 mg/kg d.w. in 23 samples of wild growing edible mushroom (Mleczek et al., 2013), from 0.007 to 0.178 mg/kg (mean 0.040 mg/kg) in nine samples of freshwater fish (roach, bream and carp) (Skibniewska et al., 2009), from 3.08 to 8.84 mg/kg d.w. in four samples of herbal teas, and from 1.16 to 2.69 mg/kg in the tea infusions (Szymczycha-Madeja et al., 2013). Ni concentrations in organic and conventional samples of carrot, celery and red beet juices (n = 39) ranged from 0.15 to 0.29 mg/kg in organic samples and from 0.14 to 0.22 mg/kg in conventional samples (Domagala-Swiatkiewicz and Gastol, 2012). In organic and conventional samples of apple, pear, black currant juices (n = 33), the Ni content ranged from 0.04 to 0.23 mg/kg in organic samples and from 0.06 to 0.22 mg/kg in conventional samples (Gastol and Domagala-Swiatkiewicz, 2012).

Ni concentrations found in 25 Portuguese red wines ranged from < 0.033 (LOD) to 1.1 mg/L (Santos et al., 2013).

In Romania, in 12 samples of milk, 10 samples of sheep cheese and in 20 samples of poultry liver, Ni concentrations ranged from 0.005 to 0.039 mg/L, from 0.002 to 0.010 mg/kg and from < 0.006 (LOQ) to 0.010 mg/kg, respectively (Ghimpeteanu, 2009; Gogoasa et al., 2006; Ghimpeteanu et al., 2012). Ni concentrations in 34 samples of fish, meat and meat products ranged from 0.082 and 0.240 mg/kg (Tudor et al., 2009a). In 118 samples of canned meat products, the Ni average content varied between 1.69 and 1.90 mg/kg in shoulder pork, ham and lunch pork and 5.39 mg/kg in pork liver paste (ranges 0.49–10.63 mg/kg) (Tudor et al., 2009b). Products in glass and china containers had higher Ni mean values than those in plastic and metallic containers.

The Ni contents in different samples of cereals (wheat, barley and oat) sampled in three contaminated soil regions of Slovakia ranged from 0.07 to 4.25 mg/kg (Mikuška et al., 2008). Concentrations in 30 samples of raw and ultra heat treated (UHT) cow milk collected during the period from 2011 to 2012 ranged from 0.25 to 1.65 mg/kg with an average value of 0.84 and 1.01 mg/kg in raw and UHT milk, respectively (Lukacova et al., 2012).

In Slovenia, Ni concentrations determined in 42 selected food products (with cocoa and soya as an ingredient, oat flakes, banana chips, hazel nuts, mussels and teas) ranged from 0.20 in raisins to 11.5 mg/kg d.w. in 100 % cocoa (Ščančar et al., 2013b). The same authors analysed the total and the speciation of Ni in dry leaves of white, green, oolong and black tea (*Camellia sinensis*) and flowers of herbal chamomile (*Matricaria chamomilla*) and hibiscus (*Hibiscus sabdariffa*) tea (Ščančar et al., 2013a). The total concentrations ranged from 1.21 to 14.4 mg/kg and during the infusion process, up to 85 % of Ni was extracted from tea leaves or flowers. Ni was found to be present in the chromatographic fraction in which quinic acid was identified by Q-TOF in all the tea infusions analysed, which had pH values between 5.6 and 6.0. The only exception was the infusion of hibiscus tea with a pH of 2.7, where results of speciation analysis showed that Ni is present in its divalent ionic form.

In Spain, the Ni content ranged from 0.41 to 1.08 mg/kg in 50 samples of seafood, meat, legumes, cheese, cereals and dried fruits (Yebra et al., 2008), from 0.001 to 0.042 mg/kg in 65 samples of vegetable and oil from Spain and Morroco (Bakkali et al., 2012), from 0.019 to 0.095 mg/kg in 170 samples of 43 different convenience and fast foods widely consumed in Spain (Cabrera-Vique et al., 2011), from 0.009 to 0.27 mg/kg in 62 samples of liver, kidney and muscle of pigs (Lopez-Alonso et al., 2007), from 0.02–0.35 mg/kg and 0.10–0.64 mg/kg respectively in 40 samples of different legumes and 56 samples of different nuts, that are widely consumed in Spain (Cabrera et al., 2003) and from 0.050 to 1.10 mg/kg in 57 varieties of cheese (Moreno-Rojas et al., 2010). In 144 samples of vegetables, fruits and rice sampled in 16 localities from the riparian zone of the Ebro River in Tarragona Province and its Delta, in Catalonia, the average Ni concentrations ranged from < 0.010 (LOD) to 0.49 mg/kg (range < 0.010–2.37 mg/kg) (Ferré-Huguet et al., 2008). In the 440 samples analysed for the total diet study of Canary Islands, Ni concentrations ranged from 0.002 mg/L in water (LOQ 0.0014 mg/L) to 2.348 mg/kg in nuts (González-Weller et al., 2012). The mean Ni content determined in a total of 360 samples (10 samples of each milk were taken monthly throughout one year) of raw milk of cow, ewe and goat were 0.015, 0.014 and 0.019 mg/kg, respectively (Amaro et al., 1998). Ni concentrations ranged from 0.015 to 0.060 mg/L in milk from organic and conventional farms in NW Spain (n = 50), and no statistically significant difference was observed between organic and conventional milks (Rey-Crespo et al., 2013).

The Ni average contents in the Swedish market study (116 foods and beverages divided into 14 food groups purchased during March-May 1999 in four cities representing the major geographical regions and population centres in Sweden) ranged between < 0.0004 mg/kg in soft drink, light beer and 0.36 mg/kg in sugar and sweets (Becker and Kumpulainen, 2011).

In the 2006 UK total diet study (TDS), Ni was detected in various food groups ranging from 0.02 mg/kg for the offal group to 3.2 mg/kg for the nuts group but the concentrations in carcase meat, poultry, oils and fats, eggs and milk were below the LODs of 0.007–0.04 mg/kg (Rose et al., 2010). These concentrations were broadly similar to those reported in the 2000 TDS (FSA, 2004). In the 1997 UK TDS, the concentrations ranged from 0.005 mg/kg in milk group to 1.8 mg/kg in the nuts group (Ysart et al., 2000). In a wide range of commercial weaning foods and formulae (n = 201), Ni was detected at concentrations at or above the LOD (0.008–0.05 mg/kg depending on sample weight taken) (FSA, 2006). The mean concentration was 0.1 mg/kg (mean range 0.035–0.463 mg/kg) and the maximum value of 0.9 mg/kg was found in a sample of porridge. In eight commercial infant foods in the UK, targeted for infants aged between six and 12 months, Ni concentrations ranged from < 0.080 (LOQ) to 0.41 mg/kg (Zand et al., 2012).

The Ni concentrations in 15 samples of chocolate drink powder purchased in the local market of Campinas, State of São Paulo, in Brazil were all < LOQ of 0.79 mg/kg (Peixoto et al., 2012). In 223 Brazilian samples of fruits (n = 89), leafy vegetables (n = 34), green vegetables (n = 74) and 'general' vegetables (n = 26), Ni content ranged from non detected (n.d.) to 0.40 mg/kg, from 0.07 to 0.70 mg/kg, from 0.10 to 0.74 mg/kg, and from 0.06 to 0.47 mg/kg, respectively (Guerra et al., 2012). In eight samples of cashew nuts from conventional and organic cultivation collected at four stages of processing (after shelling, before peeling, after peeling and packing), Ni content ranged from 0.36 to 0.68 mg/kg (mean 0.60 mg/kg) (Soares et al., 2012). In 19 different juices of seven different brands, Ni concentrations ranged from 3.9 to 30.7 µg/L (LOD 0.5 or 2 µg/L) (Tormen et al., 2011).

The Ni concentrations in 55 samples of marine food supplements sampled in Canada (31 algae products, 16 shark cartilages, five coral and three krill) ranged from 0.26 to 73 mg/kg d.w. (mean of 8 mg/kg d.w. in algae, 3 mg/kg d.w. in coral, 0.83 mg/kg d.w. in krill and 1.1 mg/kg d.w. in shark cartilages) (Leblond et al., 2008).

In India, the Ni concentrations in four species of mushrooms ranged from 0.07 to 0.15 mg/kg d.w. (Mallikarjuna et al., 2013).

In Iran, the Ni concentrations in 11 various food and agricultural products and in muscles of 24 farmed and wild rainbow trout ranged from 0.003 to 0.010 mg/kg and from n.d. to 0.998 mg/kg d.w., respectively (Fallah et al., 2011; Behbahani et al., 2013).

In Malaysia, the average Ni content in 36 chicken and quail muscles were 1.19 and 0.33 mg/kg d.w., respectively (Abduljaleel et al., 2012).

Concentrations of Ni in 180 eggs from farms in Southern Nigeria ranged from 0.01 to 2.06 mg/kg (mean of 0.86 mg/kg) (Iwegbue et al., 2012). The Ni ranges in 24 samples of commonly consumed food crops and in 12 fruits were < 0.001 (LOD)–3.13 mg/kg and < 0.001–1.76 mg/kg, respectively (Orisakwe et al., 2012).

In 15 vegetables and fruits (okra, guava, banana, potato, chili paper, onion, tomato, mint, mango, ginger, brinjal, bitter gourd, spinach, carrot) available in the markets of Hyderabad city, Pakistan, the Ni content ranged from 0.05 to 1.8 mg/kg (Ismail et al., 2011). Previously, the Ni ranges found in 88 samples of fruits and vegetables purchased from local market of Karachi were n.d.–9.05 mg/kg d.w. (Parveen et al., 2003). The mean Ni concentration in 20 muscle samples of common carp collected from River Kabul at Nowshera, Pakistan was 74.7 mg/kg (Yousafzai et al., 2012).

In six samples of black tea most commonly consumed in Saudi Arabia, the Ni content ranged from 5.63 to 11.9 mg/kg (mean 7.7 mg/kg; LOQ 2.8 µg/L) (Shaltout et al., 2013).

The Ni content in 20 samples of smoked meat (pork, beef, turkey and chicken) in Serbia ranged from 0.34 to 0.68 mg/kg (LOQ 0.34 mg/kg) (Mitič et al., 2012).

In Turkey, in local goat milk, strained and salted yoghurt (n = 3), Ni concentrations ranged from 1.38 mg/kg d.w. in raw milk to 10.1 mg/kg d.w. in salted yoghurt (Güler, 2007). In eight samples of ewe and goat milk and their yoghurt and whey products, Ni concentrations ranged from 1.21 to 2.95 mg/kg (Sanal et al., 2011). Ni concentrations ranged from 0.030 to 0.175 mg/kg in 24 commercial fruit juices (apricot, cherry, orange and peach nectars), from 2.02 to 3.55 mg/kg in 16 potato cultivars (grown at Erzurum, Turkey), from 2.30 to 5.83 mg/kg in ten samples of dried apricot and from 0.01 mg/kg in kidney to 2.08 mg/kg in meat in 12 samples of chicken products (Saracoglu et al., 2009; Uluozlu et al., 2009; Öztürk et al., 2011; Harmankaya et al., 2012).

The Ni concentrations in 11 various botanicals, 21 dietary supplements and six herbal supplements consumed in USA ranged from 0.68 to 6.82 mg/kg, from 0.33 to 15.4 mg/kg, and from 0.551 to 7.31 mg/kg, respectively (Avula et al., 2010; Bu et al., 2013). In 19 samples of acidic food of red cabbage, sauerkraut, honey, vinegar, whey cheese and wine, Ni content ranged from 0.02 to 1.1 mg/kg (Stoewsand et al., 1979).

4.1.2. Nickel in breast milk

In general, low levels of Ni are found in breast milk. Apart from one study in Turkey where the average concentration was 43.9 µg/L (n = 60) (Gürbay et al., 2012), in other studies Ni was quantified at average levels of 1.2 µg/L in USA (n = 46) and Brazil (n = 58) (Casey and Neville, 1987; Cardoso et al., 2014), 5.8 µg/L (n = 34 of colostrum samples) or 7.6 µg/L (n = 19 of mature milk samples) in Portugal (Almeida et al., 2008), 0.79 µg/L in Austria (Krachler et al., 2000), and 6.6 µg/L (n = 10) in Iraq (Hassan, 2009).

4.1.3. Nickel in drinking water

Ni concentrations in tap water can be influenced by the origin of the water (surface water, ground water, geological layer), its subsequent treatment process, piping and tap material, and stagnation time. Some evidence suggests that corrosion of steel pipes in domestic water distribution systems contributes Ni to water drawn from taps, especially during the first draw (Hoekstra et al., 2003, 2004; De Brouwere et al., 2012). According to the synthesis reports on the quality of drinking water in the

EU MS, Ni generally complied in 99 % or more of the samples taken to the limit of 20 µg Ni/L, except in four MS in the period 2002-2004 and in seven MS in the period 2005-2007 (EC, 2007, 2011). According to the review of De Brouwere et al. (2012), a small proportion of the EU population (< 5 %) is likely to be exposed to tap water exceeding the limit (parametric value) of 20 µg/L for water intended for human consumption (EU Directive 98/83/EC).

Ni concentrations in drinking-water in European countries of 2-13 µg/L have been reported (IARC, 1990; WHO, 2000). Drinking water generally contains Ni at concentrations less than 10 µg/L (Anses, 2005; Cempel and Nikel, 2006; WHO, 2007; Bertoldi et al., 2011; De Brouwere et al., 2012). Examples of Ni occurrence in drinking water are reported hereafter, while a summary of Ni in environmental water and drinking water sources is available in Section 1.

At the tap of the user in France, out of the 12 800 results on Ni extracted from the French SISE-EAUX database (Health and Environment Information System on Water) for the period January 2004 to March 2005, 98.4 % were below 20 µg/L and among the 208 cases of non-compliance reported, about 30 % were greater than 50 µg/L (Anses, 2005). At the 7 824 water company outlets, more than 99 % of the 12 300 analyses were below 20 µg/L and among the 62 cases of non-compliance reported, less than 18 % were greater than 50 µg/L.

The median Ni concentration in 164 German tap water samples was 0.486 µg/L (LOD 0.01 µg/L) (Birke et al., 2010).

In Italy, Ni median and maximum concentration in 15 tap waters collected in 2005 after 5 min flushing time were 0.6 and 2.5 µg/L (LOD 0.1 µg/L) (Cidu et al., 2011). In 10 samples of Sicilian tap waters, Ni concentrations ranged from 2.1 to 3.1 µg/L (LOD 0.155 µg/L) (Varrica et al., 2013).

The Ni concentrations in 18 tap water samples from Norway, Sweden, Finland and Iceland ranged from 0.045 to 1.59 µg/L (median 0.369 µg/L, LOD 0.01 µg/L) (Frengstad et al., 2010).

In Poland, average Ni concentrations in drinking water ranges from 3 to 7 µg/L, but it increases in vessels that contain corroded Ni plating (Duda-Chodak and Blaszczyk, 2008).

In Australia, the concentration of Ni in drinking water is typically less than 10 µg/L. In Sampleton, Australia, the mean Ni concentration in drinking water found in water samples taken between January 2002 and December 2005 was 30 µg/L (range < 10–220 µg/L) and intermittently exceeded the Australian Drinking Water Guidelines (ADWG) value for Ni of 20 µg/L (Alam et al., 2008).

In Canada, in surveys of drinking water supplies conducted between 1985 and 1988 in Northern Alberta and the Atlantic Provinces, the mean concentrations ranged from 2.1 to 2.3 µg/L and from 0.2 to 7.2 µg/L in a survey of 96 plants across Ontario, with the exception of those for Sudbury (Health Canada, 1994). Levels in drinking water in the Sudbury area sampled between 1972 and 1992 were markedly higher, with mean concentrations ranging from 26 to 300 µg/L. The median Ni concentrations in both treated and distributed provincial drinking water measured in an extensive national survey of many Canadian municipalities were ≤ 0.6–1.3 µg/L for treated water and 1.8 µg/L for distributed water, the maximum value reaching 72.4 µg/L (ATSDR, 2005). Ni levels in tap waters from British Columbia, Prince Edward Island, the Yukon, and Northwest Territories were below detection limit.

Tap water that is used for drinking purposes generally contains Ni at concentrations ranging from 0.55 to 25 µg Ni/L in the United States (ATSDR, 2005; OEHHA, 2011). In a Seattle (Washington) study, mean and maximum Ni levels in standing water were 7.0 and 43 µg/L, respectively, compared with 2.0 and 28 µg/L in running water (ATSDR, 2005). A similar result was observed in another study in which Ni levels were measured in standing tap water and in tap water after the water line had been flushed for few minutes (Thomas et al., 1999). Ni concentrations in tap water measured in the US Total Diet Study 1991–1999 ranged from 0 to 25 µg/L with a mean value of 2 µg Ni/L. Analysis of

data obtained during 1995–1997 from the National Human Exposure Assessment Study (NHEXAS) yielded median concentrations of Ni in tap water (used as drinking water) of 4.3 µg Ni/L (10.6 µg Ni/L, 90th percentile) in the Arizona study and 4.0 µg Ni/L (11 µg Ni/L, 90th percentile) in the US EPA Region 5 (Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin) study. According to the monitoring data collected by the California Department of Health Services (DHS) between 1984 and 1997, the highest, average and median concentrations of Ni in water were 540 µg/L, 26 µg/L, and 17.9 µg/L, respectively.

4.1.4. Nickel in bottled water

In a survey of the chemical composition of 571 European bottled mineral waters marketed in 23 European countries, Ni was above the LOD of 1.9 µg/L in less than 12 % of samples (median < 1.9 µg/L; 90th percentile 2.2 µg/L), and only two samples exceeded the EC limit of 20 µg/L reaching the maximum of 30.3 µg/L (Bertoldi et al., 2011). In a large scale campaign involving 1 785 samples of bottled water from 884 individual locations, Ni concentrations ranged from < 0.01 to 95 µg/L (median and 95th percentile values of 0.2 and 5.8 µg Ni/L, respectively) with six samples exceeding the EU limit of 20 µg/L (Demetriades, 2010b). Results from other studies from EU MSs are summarized in Table 6.

Table 6: Ni concentrations (in µg/L) in bottled waters from different European countries

Origin	n	LOD	Min	Max	Mean	Median	N > 20 µg/L	Reference
EU	56	0.012	0.16	14.4	-	2.46	-	Misund et al. (1999)
EU	571	1.9	-	30.3	-	< 1.9	2	Bertoldi et al. (2011)
EU	1785	0.01	< 0.01	95	-	0.2	6	Demetriades (2010b)
Croatia	14	0.01	0.059	5.28	-	-	-	Peh et al. (2010)
Estonia	5	0.005	0.112	21.6	-	-	1	Bityukova and Petersell, (2010)
Germany	908	0.01	< 0.01	26.4	1.37	0.251	5	Birke et al. (2010)
Greece	61	0.005	0.011	2.4		0.136	-	Demetriades (2010a)
Greece, France	16	-[(a)]	0.10	1.10	0.56	0.56	-	Karamanis et al. (2007)
Hungary	36	0.02	< 0.02	6.67		0.137	-	Fugedi et al. (2010)
Italy	186	0.01	< 0.01	6.62	0.41	0.13	-	Cicchella et al. (2010)
Italy, France	37	0.01	< 0.01	12	-	0.4	-	Cidu et al. (2011)
Italy	16	0.155	< 0.155	7.0	2.03	1.76	-	Varrica et al. (2013)
Norway, Sweden, Finland, Iceland	22	0.01	< 0.01	1.03	-	0.018	-	Frengstad et al. (2010)
Serbia	13	0.01	0.047	9.12	-	-	-	Petrovic et al. (2010)
Slovenia	51	-	-	49	-	-	2	Brenčič et al. (2010)
UK	67	0.1	< 0.1	4.12	-	0.723	-	Smedley (2010)

(a): not indicated.

In Turkey, the Ni content of 70 bottled waters ranged from 0.09 to 7.48 µg/L (LOD 0.003 µg/L; median and mean 0.15 and 0.51 µg/L, respectively) (Güler and Alpaslan, 2009). Previously, Ni concentrations were estimated in 69 Turkish bottled water brands between 2 and 100 µg/L (range 2–20 µg/L in 63 natural spring and mineral waters; 2–100 µg/L in six samples of drinking water) (Güler, 2007).

In Nigeria, the Ni content of 34 bottled waters ranged from 2.18 to 18.3 µg/L (Nkono and Asubiojo, 1997).

4.2. Conclusions

In general, food was reported to contain Ni at concentrations less than 0.5 mg/kg. The highest mean concentrations of Ni have been measured in wild growing edible mushrooms, cocoa or cocoa-containing products (> 10 mg/kg d.w.), beans, seeds, nuts and grains. Ni concentrations in waters and breast milk were generally below 10 µg/L.

4.3. Current occurrence results

The EFSA Evidence Management Unit (DATA Unit) published a call for available data on Ni and chromium (trivalent and hexavalent) levels in food and drinking water . European national food authorities and similar bodies, research institutions, academia, food and feed business operators and any other stakeholders were invited to submit analytical data. The data for the present assessment where provided in the framework of the annual data collection by the national authorities listed in Figure 4. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a).

By the end of April 2014, a total of 57 928 samples of food and drinking water were available in the EFSA database. Most of the samples (57 879) reported Ni data as 'Nickel' without further information, while for 49 samples the data were reported as 'Nickel and derivatives'. No data on Ni speciation were reported. For the dietary exposure calculations described in this scientific opinion, all samples were considered as reporting Ni. Approximately 63 % of the samples were reported as drinking water and 37 % as food samples. Samples were mostly collected in Germany (79 %), Slovakia (11 %) and Cyprus (5 %), between 2000 and 2012.

In order to guarantee an appropriate quality of the data used in the exposure assessment the initial dataset was carefully evaluated applying several data cleaning and validation steps (e. g. exclusion of duplicates and samples without complete information). Following this approach 3 784 samples were excluded as they reported neither LOD nor LOQ. Four samples of drinking water for which the analytical method reported was described as 'Organoleptic (sensoric) test of foods' were excluded from the final dataset. Likewise, when the information on the sampling strategy was described as 'suspect samples', the samples were excluded from the final dataset since they do not represent random sampling (605 samples). Food samples codified as 'Grain as crops', which refer to unprocessed grains of undefined end-used, were also excluded (114 samples). Finally, only the data from the last 10 years (2003 onwards) were considered for the exposure calculations (2 902 samples excluded).

4.3.1. Data collection on food (including drinking water)

After the first quality assessment of the analytical data, a total of 50 519 samples of food and drinking water were available with data reported on Ni. In the FoodEx classification system (EFSA, 2011a) different types of water (bottled water, tap water, water ice and well water) are grouped under the generic name 'Drinking water'. Following the European legislation (Council Directive 98/83/EC and Commission Directive 2003/40/EC), tap water, water ice and well water would be included under the term 'Water intended for human consumption' while bottled water (still and carbonated) would belong to 'Natural mineral waters'. The dataset for drinking water consisted of 31 574 samples, while the food dataset was made up of 18 945 samples. As shown in Figure 4, samples were collected in 15 different European countries, most of them in Germany (80 %). The sampling of the different food commodities and drinking water samples was well distributed across the years, from 2003 to 2012 (Figure 5).

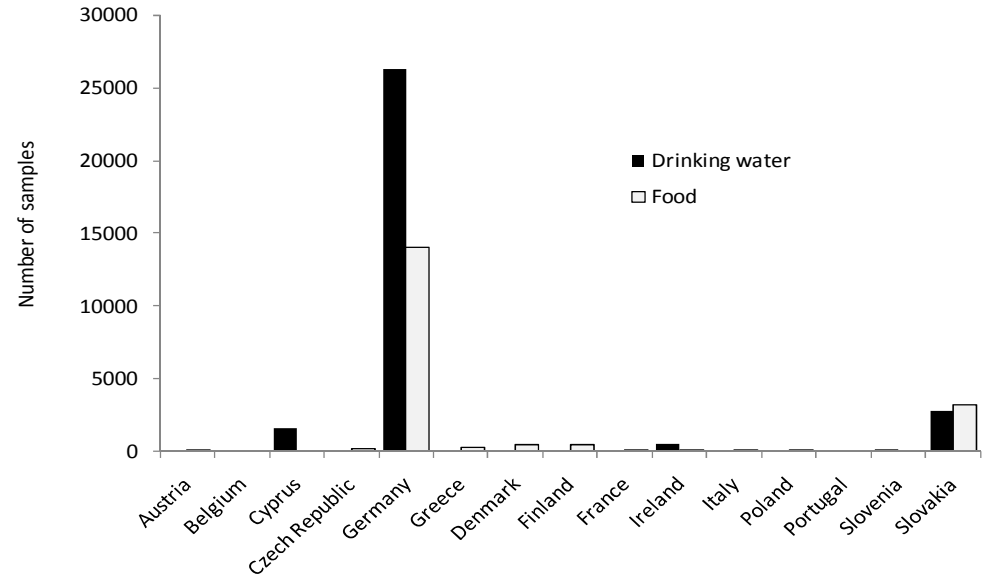


Figure 4: Distribution of food and drinking water samples analysed for nickel across different European countries

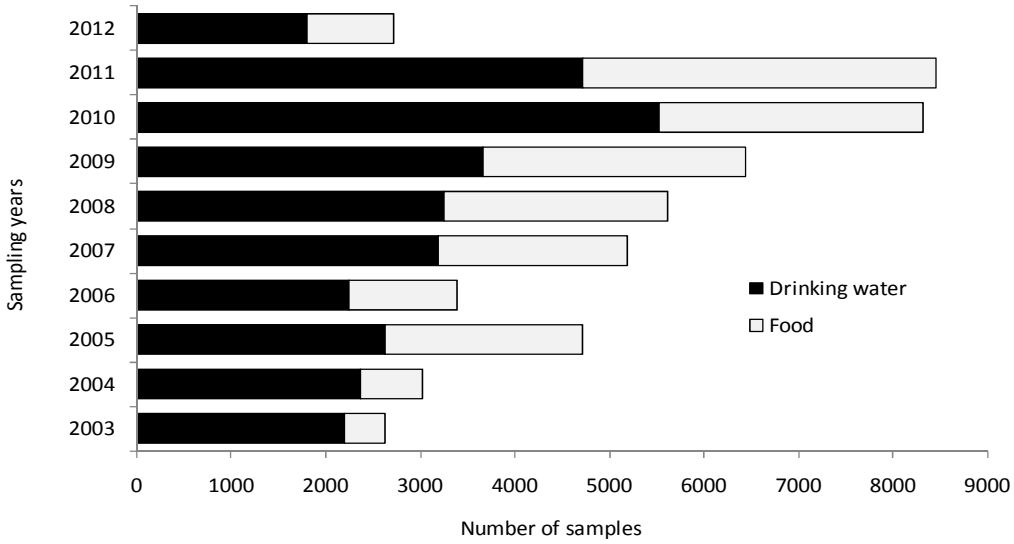


Figure 5: Distribution of food and drinking water samples analysed for nickel over the sampling years

All analytical results were expressed as whole weight, except 490 results, for which no information was provided. After a careful evaluation of these data (occurrence values and FoodEx classification), they were kept in the final dataset with their analytical results taken as reported. Another group of 220 samples were reported as 'pooled samples'. Pooled samples refer to different foods from the same or similar food groups that are mixed to make a unique sample. In this particular case, most of the pooled samples were made up of 12 individual samples codified mainly at FoodEx level 3. Pooling food samples is one of the characteristics of Total Diet Studies (TDS) together with the analysis of food samples as consumed (EFSA, 2011b). For this particular contaminant, the use of TDS samples might imply higher occurrence levels as compared to the other available samples, due to the possible leaching from stainless steel cookware used during food preparation (Kamerud et al., 2013). However, after a comprehensive assessment of the occurrence values and comparison with those present in the

other samples (within the same FoodEx category), the pooled samples were kept in the final dataset as reported.

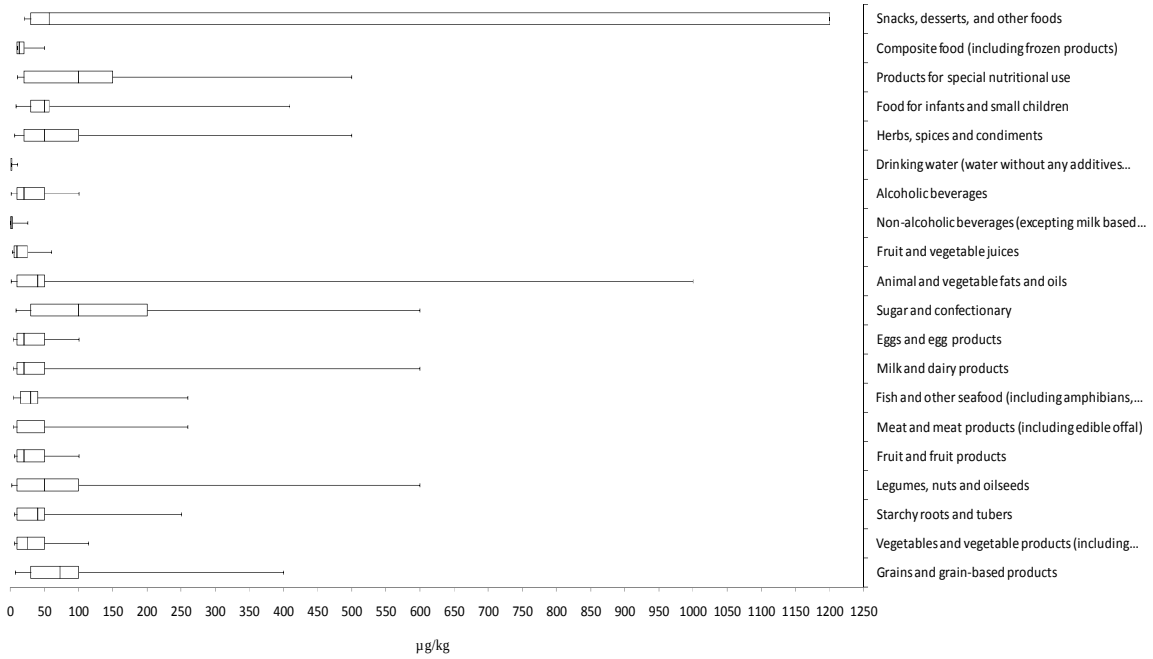
Analysis of extreme values

As a last step of the data quality control, an outlier analysis was performed using the Tukey's method (Tukey, 1977), which identifies as a statistical outlier a value greater than the 75th percentile plus 1.5 times the inter-quartile distance, or less than the 25th minus 1.5 times the inter-quartile distance. Those samples identified as potential outliers were checked to confirm the absence of errors relative to reporting units, expression of results, etc. The data providers were asked to confirm these data. Several analytical results were corrected by the data providers but in some cases no answer was obtained. Among the potential outliers that were not confirmed by the data providers there were thirteen samples of regular beer that reported Ni concentrations between 5 200 µg/L and 14 300 µg/L, one sample of carbonated water (10 800 µg/L) and one sample of pork liver with a reported value of 172 000 µg/kg. The presence of high levels of Ni in beer was not identified in the literature and, in addition, the remaining samples of regular beer were mainly left-censored data (83 %, n = 159). Moreover, the information provided on these samples does not allow for any relationship between the high levels of Ni and specific brands, sampling countries or the containers used (can, bottle) to be established. Based on this, the CONTAM Panel decided to exclude the thirteen samples of regular beer from the final dataset.

4.3.2. Analytical methods used

In more than 70 % of the cases the data providers did not report information on the analytical method used to analyse the presence of Ni. Among the samples that provided this information, the most reported analytical methods were inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS), that represented 54 % and 42 % of the methods reported, respectively. Other analytical methods reported were inductively coupled plasma atomic emission spectroscopy (ICP-AES) (3 %), electrochemical tests such as voltammetry and polarography (1 %), and spectroscopy methods (0.2 %).

A very wide range of limits of quantification (LOQs) was observed among the methods used to analyse the different food and drinking water samples (Figure 6). LOQs ranged between 0.001 µg/kg and 6 800 µg/kg. As seen in Figure 6, relatively high LOQs were reported for the food group 'Snacks, desserts, and other foods'. Within this food group, all the samples that reported analytical methods with low sensitivity (1 200 µg/kg) refer to the subgroup 'Other foods' which includes those foods that cannot be codified under other food groups, and that hardly have an impact on the dietary exposure estimations. Particularly, for ICP-MS the LOQs reported for food samples varied between a minimum of 0.002 µg/kg for 'Alcoholic beverages' and a maximum of 2 500 µg/kg for 'Products for special nutritional use'. The highest sensitivity for ICP-MS was reported for the analysis of drinking water samples with a minimum LOQ of 0.001 µg/L and a maximum of 500 µg/L. The same sensitivity was reported for AAS for the analysis of drinking water (LOQ = 0.001 µg/L), while for food samples the lowest LOQ was reported for the analysis of 'Fish and seafood' and 'Sugar and confectionery' (1 µg/kg). For more details see Appendix B. Regarding the analytical methods used to analyse the presence of Ni in drinking water, 5 874 samples were analysed using methods with LOQ > 4 µg/L (18.6 % of the total). Amongst these samples, 37 of them used methods that reported LOQ > 20 µg/L, the parametric value/maximum limit specified in the legislation. Most of these samples corresponded to bottled water (92 %). The value of 4 µg/L, taken as reference, is derived from the performance characteristics specified in the Council Directive 98/83/EC and the Commission Directive 2003/40/EC, where a limit of detection (LOD) of 2 µg/L is specified for the analytical methods used to analyse Ni in water. All the 5 874 samples (91 % left-censored data) were excluded from the final dataset since the analytical method used was considered not fit for purpose.



Box-plot: whiskers at 5th percentile and 95th percentile, box at 25th percentile and 75th percentile with line at 50th percentile

Figure 6: Distribution of LOQs among the analytical results across the different samples at FoodEx level 1

The left-censored data (analytical data below LOD/LOQ) accounted for 69 % of the analytical results. LOQs were reported for all the analytical results; in almost half of the analytical data (44 %) only the LOQ was provided. Among those food groups with the highest amount of left-censored data were 'Drinking water' (89 %), 'Eggs and egg products' (74 %) and 'Alcoholic beverages' (68 %). On the other side, the food group with the highest number of quantified data was 'Legumes, nuts and oilseed', where only 3 % of the analytical data were left-censored (Figure 7).

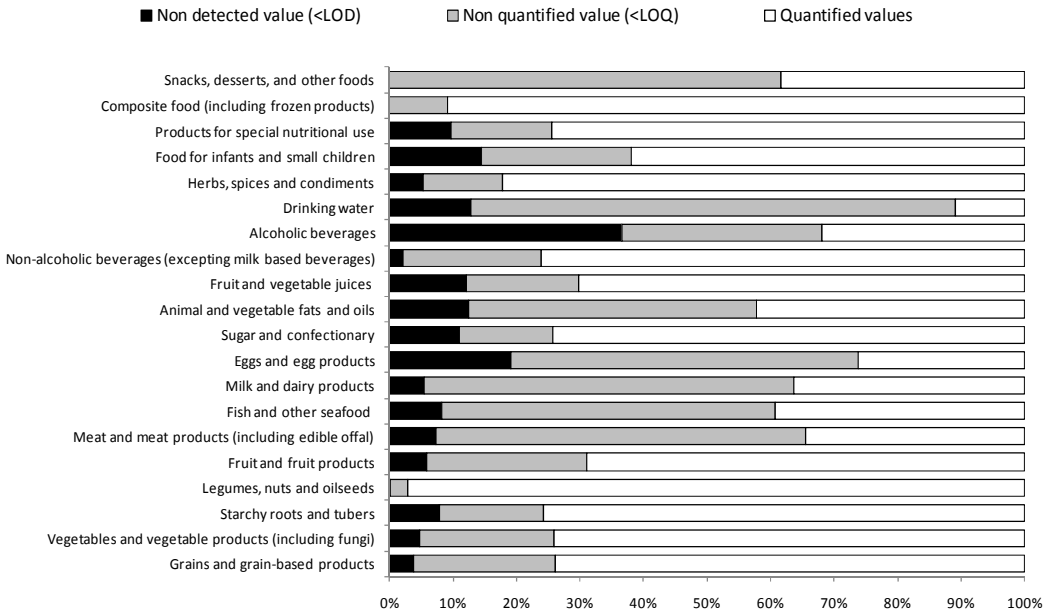


Figure 7: Percentage of analytical results below LOD, below LOQ and quantified in the final food dataset across the different food categories (FoodEx Level 1)

4.3.3. Occurrence data by food category (including drinking water)

The left-censored data were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009). The same method is indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower-bound (LB) and upper-bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). At the LB, results below the LOQ and LOD were replaced by zero; at the UB the results below the LOD were replaced by the LOD and those below the LOQ were replaced by the value reported as LOQ.

The presence of a high percentage of left-censored data together with high left-censoring limits can provoke substantial differences between LB and UB scenarios increasing the uncertainty associated to the dietary exposure estimations. Based on this fact, the Ni occurrence data were thoroughly evaluated at the different FoodEx levels. Three food groups were identified as particularly influenced by the presence of left-censored data and high left-censoring limits. These food groups were 'Fermented milk', 'Food for infants and small children' and 'Beer', foods that are regularly consumed and that, therefore, could play an important role on the dietary exposure.

Regarding 'Fermented milk' (FoodEx level 2) only 90 samples were available, 81 of them reporting left-censored data. Among the quantified samples the average concentration of Ni was 49.3 µg/kg (maximum = 135 µg/kg). Based on these values, it was decided to exclude from the final dataset 32 samples with reported LOQ of 600 µg/kg, all non-quantified samples. By doing this, the occurrence value changed from 4.9–262.3 µg/kg (LB-UB) to 7.7–76.0 µg/kg (LB-UB). The samples of 'Fermented milk' included in the final dataset possessed LOQs that ranged between 5 µg/kg and 150 µg/kg.

Within the food group 'Food for infants and small children' (FoodEx level 1) important differences were observed between LB and UB in food categories such as 'Fruit juice and herbal tea for infants and young children' (LB = 25.5 µg/kg, UB = 173.4 µg/kg) and 'Ready-to-eat meal for infants and young children' (LB = 33.0 µg/kg, UB = 165.4 µg/kg). In these food categories eight non-quantified samples reported LOQs of 1 000 µg/kg (seven) and of 6 800 µg/kg (one). Based on the occurrence values of the quantified samples it was decided to exclude the samples with LOQ ≥ 1 000 µg/kg. After excluding these samples, the occurrence values in the food categories 'Fruit juice and herbal tea for infants and young children' and 'Ready-to-eat meal for infants and young children' were 29.8–35.6 µg/kg (LB-UB) and 36.0–91.2 µg/kg (LB-UB), respectively.

A total of seven samples of 'Beer, regular' (FoodEx level 3) were also excluded from the final dataset. These samples were all left-censored data reporting an LOQ of 5 000 µg/L, a value far higher than the Ni average concentration calculated for the quantified samples of beer (5.8 µg/L, n = 27). Among the samples of regular beer considered in the dietary exposure estimations (n = 152) the LOQs varied between 0.002 µg/L and 100 µg/L, with a median value of 20 µg/L. After excluding these 47 samples, the final dataset was composed of 44 585 samples of food and drinking water (25 700 of drinking water and 18 885 of food).

Based on FoodEx classification, all groups at FoodEx level 1 were represented (Table 7). After the most represented group, 'Drinking water' with 25 700 samples available, the food groups with the highest number of samples were 'Grain and grain-based products' and 'Vegetables and vegetable products (including fungi)' with 4 291 and 3 738 samples, respectively. On the contrary, only 46 samples were reported as 'Non-alcoholic beverages'.

The food groups with the highest levels of reported Ni were 'Products for special nutritional use', 'Legumes, nuts and oilseeds' and 'Sugar and confectionery'. The samples of 'Drinking water' reported the lowest mean values of Ni among the different groups at FoodEx level 1.

A total of 471 samples were reported as 'Products for special nutritional use'. In many cases, for this food category specific details on the type of food product are missing and the comparison with published values in the literature is somehow difficult. Mean reported values at FoodEx level 1 were 1 999–2 051 µg/kg (LB-UB), being the subgroup 'Mineral supplements' the one with the highest mean concentration with 4 707–4 728 µg/kg (LB-UB, n = 45). Other food products that reported high levels of Ni were 'Plant formula extracts' with levels between 3 844 µg/kg and 3 860 µg/kg. Several studies found high amounts of Ni in diverse types of food supplements as described in Section 4.1.1.

In the group 'Legumes, nuts and oilseeds' (n = 1 218) the mean reported concentrations of Ni were 1 862–1 880 µg/kg (LB-UB). The relatively mean high concentration reported for Ni in this group agrees with published studies that describe legumes and nuts as one of the main sources of Ni in the diet (Nielsen and Flyvholm, 1984; Cabrera et al., 2003). Nuts and oilseeds were also mentioned in several TDS (carried out in France and the UK) as one of the food groups containing the highest levels of Ni (Leblanc et al., 2005; Rose et al., 2010, Arnich et al., 2012). Within this group, the subgroup 'Dried beans' contained the highest mean levels of Ni (3 055–3 077 µg/kg, LB-UB), especially 'Soya beans' (4 624–4 685 µg/kg, LB-UB) and 'Peanuts' (3 537–3 569 µg/kg, LB-UB).

The high mean levels reported for the food group 'Sugar and confectionery' (1 504–1 586 µg/kg, LB-UB, n = 1 170) were mainly due to the high levels quantified in the subgroup 'Chocolate (Cocoa) products' (3 231–3 236 µg/kg, LB-UB, n = 490). High concentrations of Ni are also described in the literature for cocoa and chocolate products (Flyvholm et al., 1984; Smart and Sherlock, 1987; Leblanc et al., 2005; Arnich et al., 2012; Ščančar et al., 2013b).

Overall, the food group 'Vegetables and vegetable products (including fungi)' reported relatively low concentrations of Ni, in many cases below 100 µg/kg (Appendix B1). However, there were two food subgroups, 'Cocoa beans and cocoa products (solid)' and 'Tea and herbs for infusion (solid)' that reported high values of Ni that influenced the mean occurrence values reported for 'Vegetables and vegetable products (including fungi)' at FoodEx level 1 (Table 7). The concentration of Ni was particularly high in 'Cocoa beans and cocoa products (solid)' with mean values of 9 528 µg/kg (LB=UB, n = 238) while in 'Tea and herbs for infusion (solid)' a mean value of 761–762 µg/kg (LB-UB, n = 105) was reported (Appendix B1). Relatively high content of Ni is commonly reported in the literature in tea and herbs for infusion (see Section 4.1.1). The food group 'Starchy roots and tubers' was mainly composed of 'Main-crop potatoes' with a mean reported value of 264–266 µg/kg (LB-UB, n = 205), and by a food category combining unspecified potatoes and potato products together with other defined potato products (French fries, potato flakes, etc.). The mean Ni concentration in this group, referred as 'Other potatoes and potato products', was 44–70 µg/kg (LB-UB, n = 279) (Appendix B1).

For 'Drinking water' the reported values for the presence of Ni were in general rather low, resulting in mean values of 1.0–2.0 µg/L (LB-UB, n = 25 700). When breaking down these samples into the different subgroups (unspecified drinking water, unspecified bottled water, carbonated mineral water, still mineral water, tap water, water ice (for consumption) and well water) the highest mean value was reported for carbonated mineral water (LB = 7.0 µg/L, UB = 8.0 µg/L, n = 2 363). The mean value reported for carbonated mineral water was undoubtedly driven by the presence of one sample that reported a concentration of 10 800 µg/L; without this particular sample the occurrence value would be in line with those reported for the other types of water.

Taking into account the existing legislation for 'water intended for human consumption' and 'natural mineral waters' (EU Council Directive 98/83/EC and Commission Directive 2003/40/EC, respectively) a total of 114 samples reported Ni concentrations above the parametric value/maximum limit of 20 µg/L. These samples corresponded to unspecified bottled water (n = 13), still mineral water (n = 4), carbonated mineral water (n = 54), tap water (n = 12), and unspecified drinking water (n = 31).

In Appendix B1 is shown a more detailed description of the occurrence values selected to calculate the dietary exposure to Ni, and how the samples were grouped before the exposure estimations were

carried out. As an example of grouping, 'Cocoa beans and cocoa products (solid)' and 'Tea and herbs for infusion (solid)' were grouped within the group 'Non alcoholic beverages (excepting milk based beverages)' when estimating dietary exposure since these food commodities are mainly consumed as beverages.

Different assumptions were done during the preparation of the occurrence data. When food categories were not represented they were, when possible, assigned an occurrence value derived from similar food commodities. In general, when less than 10 samples were reported for one specific food group, the average occurrence value of all samples contained in the immediate upper FoodEx level was used. Dilution factors were also used to match the occurrence values reported in dry samples with their respective liquid consumption amounts. An average dilution factor of 18 was used to match occurrence value in coffee beans with the different type of coffees, except for 'coffee espresso' where the dilution factor was 7 and for 'instant coffee' where it was 63. Other dilution factors used were 100 for tea and herbal leaf varieties, 60 for cocoa powder, and 8 for follow-on and infant formulae (EFSA, 2011c, d; USDA, 2013). More than 98 % of the eating occasions present in the EFSA Comprehensive European Food Consumption Database was covered by the occurrence data.

Table 7: Summary statistics for nickel concentration (µg/kg) with the different samples aggregated at FoodEx level 1 (detailed description of the occurrence values selected at the appropriate FoodEx level to calculate the dietary exposure to nickel is shown in Appendix B1). Values were rounded off to the nearest whole number (0 decimal places).

	n	LC (%)	LB/UB	Concentration (µg/kg)					
				Mean	P5	P25	P50	P75	P95
Grains and grain-based products	4291	26	LB	**271**	0	0	136	290	1 069
			UB	**321**	30	100	180	335	1 078
Vegetables and vegetable products (including fungi)	3738	26	LB	**742**	0	0	52	150	9 250
			UB	**753**	9	32	56	159	9 250
Starchy roots and tubers	664	24	LB	**123**	0	10	44	94	690
			UB	**150**	14	35	58	168	690
Legumes, nuts and oilseeds	1218	3	LB	**1862**	80	607	1 154	2055	7 000
			UB	**1880**	140	630	1 154	2055	7 000
Fruit and fruit products	966	31	LB	**68**	0	0	38	75	210
			UB	**91**	9	30	50	86	300
Meat and meat products (including edible offal)	2169	66	LB	**191**	0	0	0	46	310
			UB	**239**	10	20	50	90	500
Fish and other seafood	718	61	LB	**77**	0	0	0	50	330
			UB	**112**	12	29	40	70	390
Milk and dairy products	631	62	LB	**71**	0	0	0	40	435
			UB	**93**	9	10	25	81	488
Eggs and egg products	115	74	LB	**38**	0	0	0	10	179
			UB	**57**	6	10	30	50	179
Sugar and confectionery	1170	26	LB	**1 504**	0	0	540	3 033	5 170
			UB	**1 586**	30	230	705	3 033	5 170
Animal and vegetable fats and oils	363	58	LB	**315**	0	0	0	50	360
			UB	**378**	8	10	50	200	500
Fruit and vegetable juices	505	30	LB	**35**	0	0	15	39	102
			UB	**58**	7	11	24	50	120
Non-alcoholic beverages (excepting milk based beverages)	46	24	LB	**32**	-	2	7	13	-
			UB	**35**	-	6	9	14	-
Alcoholic beverages	892	69	LB	**28**	0	0	0	12	70
			UB	**71**	1	10	20	30	150
Drinking water	25700	89	LB	**1**	0	0	0	0	2
			UB	**2**	0	1	1	1	3

Table 7: Summary statistics for nickel concentration (µg/kg) with the different samples aggregated at FoodEx level 1 (detailed description of the occurrence values selected at the appropriate FoodEx level to calculate the dietary exposure to nickel is shown in Appendix B1). Values were rounded off to the nearest whole number (0 decimal places) (continued).

	n	LC (%)	LB/UB	Concentration (µg/kg)					
				Mean	P5	P25	P50	P75	P95
Herbs, spices and condiments	481	18	LB	**1 259**	0	83	560	1 799	4 640
			UB	**1 277**	31	120	582	1 799	4 640
Food for infants and small children	309	45	LB	**126**	0	0	60	140	500
			UB	**152**	20	50	70	158	500
Products for special nutritional use	471	26	LB	**1 999**	0	0	321	1 930	9 100
			UB	**2 051**	30	117	409	2 050	9 100
Composite food (including frozen products)	65	9	LB	**181**	0	55	81	140	490
			UB	**184**	27	55	81	140	490
Snacks, desserts, and other foods	73	62	LB	**111**	0	0	0	52	280
			UB	**430**	30	48	82	1 200	1 200

n: number of samples; LC: left-censored; LB: lower bound; UB: upper bound; P5/P25/50/75/95: 5th/25th/50th/75th/95th percentile.

5. Food consumption

5.1. EFSA's Comprehensive European Food Consumption Database

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built in 2010 based on information provided by EU Member States and the food consumption data for children obtained through an EFSA Article 36 project (Huybrechts et al., 2011). The Comprehensive Database version 1 contains results from a total of 32 different dietary surveys carried out in 22 different Member States covering more than 67 000 individuals (EFSA, 2011b). The Comprehensive Database includes individual food consumption data concerning infants (two surveys from two countries), toddlers (eight surveys from eight countries), children (16 surveys from 14 countries), adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries), elderly (nine surveys from nine countries) and very elderly (eight surveys from eight countries).

Within the dietary studies, subjects were classified in different age classes as follows: Infants (< 12 months old), Toddlers (≥ 12 months to < 36 months old), Other children (≥ 36 months to < 10 years old), Adolescents (≥ 10 years to < 18 years old), Adults (≥ 18 years to < 65 years old), Elderly (≥ 65 years to < 75 years old) and Very elderly (≥ 75 years old).

The CONTAM Panel considered that both chronic dietary and acute exposure to Ni had to be assessed. As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b), dietary surveys with only one day per subject were only considered for acute exposure as they are not adequate to assess repeated exposure. Similarly, subjects who participated only one day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Thus, for chronic exposure assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries (Appendix C1). Six additional dietary surveys with only one day per subject from six different countries (covering all age classes except infants) were considered for acute exposure assessment (Appendix C1). In the table, the number of available days for each age class used in the acute exposure assessment is described beside the number of subjects available for the chronic exposure assessment.

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, it should be pointed out that different methodologies were used between surveys to collect the data and thus direct country-to-country comparisons can be misleading. Similarly to what is described for the occurrence data,

consumption records are also codified according to the FoodEx classification system. Further details on how the Comprehensive Database is used are published in the Guidance of EFSA (2011b).

6. Exposure assessment in humans

6.1. Previously reported exposure assessments

Several studies have evaluated the dietary exposure to Ni in European populations in the past. In France, the last published TDS found a mean dietary exposure to Ni (at the middle bound approach) of 2.33 µg/kg b.w. per day in adults (18–79 years) and 3.83 µg/kg b.w. per day in children (3–17 years) (Arnich et al., 2012). At the 95th percentile, exposure estimates were 3.76 µg/kg b.w. per day in adults and 7.44 µg/kg b.w. per day in children. As compared with the previous French TDS (Leblanc et al., 2005), the estimated dietary exposure to Ni is around 25–50 % higher. In UK, TDS have also been used to estimate dietary exposure to Ni in different populations. In the most recent TDS published, mean and 97.5th percentile dietary exposures to Ni in adults (LB-UB) were 1.49–1.63 µg/kg b.w. per day and 3.01–3.08 µg/kg b.w. per day, respectively (Rose et al., 2010). In toddlers (1.5–4.5 years) and young people (4–18 years) the estimates were higher, with mean exposure of 4.17–4.87 µg/kg b.w. per day and 2.62–3.05 µg/kg b.w. per day, respectively. High level (97.5th percentile) dietary exposure to Ni was 7.54–8.32 µg/kg b.w. per day in toddlers and 5.27–5.82 µg/kg b.w. per day in young people. Population exposures to Ni in previous UK TDS have been relatively stable since 1982 with similar values to those estimated in the 2006 TDS. Beyond TDS, other studies have also reported the intake of Ni in diverse European populations. In the area of Gubbio (Italy) different methods were used to calculate the intake of Ni in a group of 44 subjects (21 men and 23 women). The chemical analysis of duplicate portions was selected as the preferable method, and the average intake of Ni reported as 165.7 µg/day in women and 222.3 µg/day in men (Alberti-Fidanza et al., 2003). In Catalonia (Spain), the amount of Ni present in a selected group of food items was analysed and combined with consumption data from the local population; estimated dietary intake of Ni was 138 µg per day with pulses being the main contributor (Bocio et al., 2005). A very recent duplicate diet study in the same region showed slightly lower intakes, with a dietary intake of Ni in the adult population of 109 µg/day (Domingo et al., 2012). In another Spanish region (Canary Island), the estimated total intake of Ni in the adult population was 93 µg/day (Gónzalez-Weller et al., 2012). In Sweden an estimation of the dietary exposure to different mineral elements using market basket diets was carried out in 1999. The estimated exposure to Ni was reported as 90 µg per day; food items such as coffee, tea and drinking water were not included in the study (Becker and Kumpulainen, 2011). Some other studies published in the literature that covered the intake of Ni through the consumption of only certain food groups (vegetables, fruits) are not discussed in this section. Although Ni is present in most foods, the main contributors to the dietary exposure to Ni were overall reported as being beverages, miscellaneous cereals, pulses, chocolate and fruits. There is a general agreement that drinking water hardly contributes to the exposure to Ni (MAFF, 1985), although in some studies such as the second French TDS water represented as average 8 % of the total contribution in adults (Arnich et al., 2012) (Table 8).

Table 8: Summary of the most recent dietary exposure assessments carried out in different European countries

Country	Mean adult exposure	High adult exposure	Mean children exposure	High children exposure	Reference
France	94 µg/day[(g)]	166 µg/day[(h)]	92 µg/day[(i)]	174 µg/day[(h)]	Leblanc et al. (2005)
Italy	165.7 µg/day[(a)] 222.3 µg/day[(b)]	484.7 µg/day[(a),(c)] 480.3 µg/day[(b),(c)]	-	-	Alberti-Fidanza et al. (2003)
Italy	361.1 µg/day	764.2 µg/day[(c)]	-	-	Turconi et al. (2009)
Spain	138.3 µg/day	-	-	-	Bocio et al. (2005)
Spain	109 µg/day	-	-	-	Domingo et al. (2012)
Spain	93 µg/ day	-	-	-	Gónzalez-Weller et al. (2012)

Table 8: Summary of the most recent dietary exposure assessments carried out in different European countries (continued)

Country	Mean adult exposure	High adult exposure	Mean children exposure	High children exposure	Reference
Sweden	90 µg/day	-	-	-	Becker and Kumpulainen. (2011)
France	2.33 µg/kg b.w. per day(d)	3.76 µg/kg b.w. per day in adults(e)	3.83 µg/kg b.w. per day in children(f)	7.44 µg/kg b.w. per day(e)	Arnich et al. (2012)
Germany	-	-	5.59 µg/kg b.w. day(m)	12 µg/kg b.w. per day(e)	Wittsiepe et al. (2009)
United Kingdom	1.49-1.63 µg/kg b.w. per day(j)	3.01-3.08 µg/kg b.w. per day(h)	4.17-4.87 µg/kg b.w. day(k) 2.62-3.05 µg/kg b.w. per day(l)	7.54-8.32 µg/kg b.w. per day(k),(h) 5.27-5.82 µg/kg b.w. per day (l)	Rose et al. (2010)

(a): Women.
(b): Men.
(c): Maximum exposure.
(d): Adults refer to individuals aged 18–79 years.
(e): 95th percentile.
(f): Children refer to individuals aged 3–17 years.
(g): Adults refer to individuals aged 15 years or more.
(h): 97.5th percentile exposure.
(i): children refer to individuals aged 3–14 years.
(j): Adults refer to individuals aged 18–64 years, LB-UB estimations.
(k): Toddlers (aged 1.5–4.5 years) LB-UB estimations.
(l): Young people (aged 4-–-18 years), LB-UB estimations.
(m): Children aged 48–63 months

6.2. Chronic dietary exposure to nickel

For calculating the chronic dietary exposure to Ni, food consumption and b.w. data at the individual level were accessed in the Comprehensive Database. Occurrence data and consumption data were linked at the lowest FoodEx possible. In addition, the different food commodities were grouped within each food category to better explain their contribution to the total dietary exposure to Ni. For each country, exposure estimates were calculated per dietary survey and age class (see Section 5.1). Chronic exposure estimates were calculated for 26 different dietary surveys carried out in 17 different European countries. Not all countries provided consumption information for all age groups, and in some cases the same country provided more than one consumption survey.

6.2.1. Mean and high chronic dietary exposure

The mean and the high (95th percentile) chronic dietary exposures were calculated by combining Ni mean occurrence values for food and drinking water samples collected in 15 countries (pooled European occurrence data) with the average daily consumption for each food at individual level in each dietary survey. Minimum, median and maximum exposure estimates across dietary surveys and age groups are reported in Table 9. Detailed mean and 95th percentile dietary exposure estimates calculated for each of the 26 dietary surveys are presented in Appendix C2. Mean chronic dietary exposure to Ni across the different dietary surveys and age classes ranged from 2.0 µg/kg b.w. per day (minimum LB, 'Elderly') to 13.1 µg/kg b.w. per day (maximum UB, 'Toddlers'). The 95th percentile dietary exposure ranged from 3.6 µg/kg b.w. per day (minimum LB, 'Elderly') to 20.1 µg/kg b.w. per day (maximum UB, 'Toddlers').

The highest dietary exposure to Ni was observed in the age classes 'Toddlers' and 'Other children'. The adult population showed, in general, lower exposure than the young population.

Table 9: Summary statistics of the chronic exposure assessment (µg/kg b.w. per day) to Ni across European dietary surveys. Estimates were rounded to one decimal place.

Mean dietary exposure (µg/kg b.w. per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	Median	Max	Min	Median	Max
Infants	3.3	-[a]	4.1	5.6	-[a]	6.3
Toddlers	5.3	7.4	11.0	7.3	10.3	13.1
Other children	4.9	6.7	8.2	5.9	8.6	9.9
Adolescents	2.7	3.5	4.9	3.4	4.1	5.9
Adults	2.2	2.7	3.0	2.7	3.4	3.6
Elderly	2.0	2.5	2.5	2.6	3.0	3.2
Very Elderly	2.2	2.4	2.7	2.8	3.1	3.2
95th percentile dietary exposure[b] (µg/kg b.w. per day)						
	Lower bound (LB)			Upper bound (UB)		
	Min	Median	Max	Min	Median	Max
Infants	8.0	-[c]	-[c]	-[c]	-[c]	12.3
Toddlers	8.7	-[a]	14.7	10.8	-[a]	20.1
Other children	9.1	12.3	16.5	11.3	14.7	18.2
Adolescents	5.6	7.3	10.7	5.9	8.0	12.3
Adults	3.7	5.1	6.1	4.7	5.8	6.9
Elderly	3.6	4.3	4.8	4.4	5.3	5.8
Very Elderly	4.0	-[a]	4.8	4.9	-[a]	5.7

b.w.: body weight; LB: lower bound; UB: upper bound
(a): Not calculated since estimates were only available from less than six dietary surveys.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(c): Not calculated since estimates were only available from one dietary survey.

Although sometimes difficult to compare due to the different age grouping, the estimated dietary exposure to Ni is overall higher than that reported in the literature (see Section 6.1). Different factors could explain this fact. First, in this scientific opinion there was an extensive coverage on the levels of Ni in food with almost 20 000 occurrence data available, and the different food categories were well represented and with an appropriate number of samples. These data allowed covering 98 % of the consumption data reported in the different dietary surveys. Moreover, the number of occurrence data also permitted a much more detailed food classification (192 different food groups) as compared to the studies published in the literature. In certain cases, an excessive grouping could lead to a dilution effect in the Ni levels in specific key foods. For example, high occurrence values in chocolate were masked within the more general food group 'Sugar and confectionery'. This smothering effect is particularly observed in TDS (EFSA, FAO and WHO, 2011). Further explanation for the divergence between estimated exposure levels and those published in the literature may be that some key foods were excluded in certain studies (Becker and Kumpulainen, 2011) or in the different methods followed to gather the consumption data

6.2.2. Contributions of different food groups to chronic exposure to nickel

Dietary exposure to Ni as well as the average contribution of the different foods is presented divided by age class and individual dietary survey. The contribution is shown using LB estimations; contributions under UB scenario are mentioned in the text whenever they notably differ from those calculated at the LB scenario. It is important to mention that some dietary surveys (DIPP and FINDIET 2007) reported the consumption data at a disaggregated level (e.g. reporting the amount of flour instead of the amount of bread), which could have an influence on the contribution of specific food categories to the dietary exposure to Ni.

Before calculating dietary exposure, the available foods were grouped at FoodEx level 1 to show their contribution to the total exposure to Ni (Appendix B1). Foods codified under several food categories were grouped under only one food category. This was the case for tea, coffee and cocoa (described as 'Vegetables and vegetable products' but also as 'Non-alcoholic beverages'); they were all grouped as 'Non-alcoholic beverages' when describing their contribution to the dietary exposure to Ni.

6.2.2.1. Infants and toddlers

Dietary exposure in infants was evaluated in only two dietary surveys. Therefore, the interpretation of the results should be done very carefully, even more considering that one of the surveys covers only 16 subjects. Mean dietary exposure to Ni in the 'Infants' ranged between 3.3 µg/kg b.w. per day and 6.3 µg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure for the single qualifying study was 8.0 µg/kg b.w. per day (LB) and 12.3 µg/kg b.w. per day (UB).

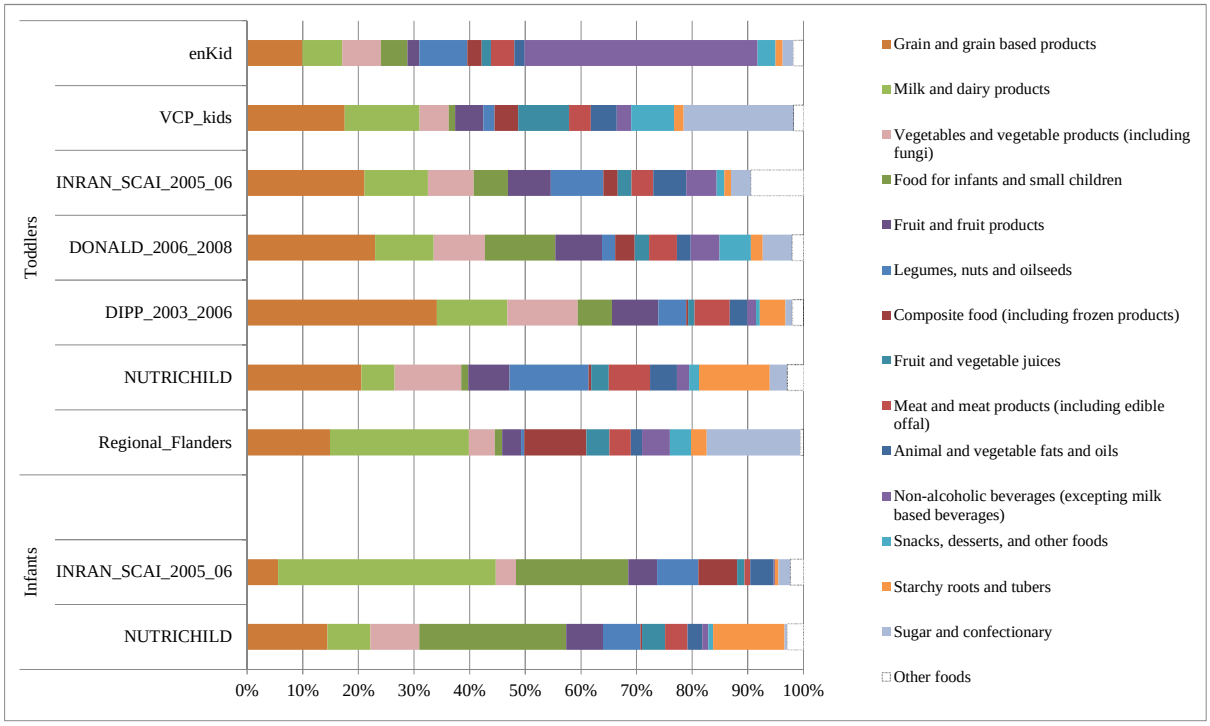
In 'Infants', the main contributors to the exposure to Ni were 'Food for infants and small children', 'Milk and dairy products', 'Grain and grain-based products' and 'Starchy roots and tubers'. The contribution of human milk to the exposure to Ni was not considered since no reported occurrence data were available. Data in the literature indicate that, in general, low levels of Ni are found in breast milk. Apart from one study in Turkey where the average concentration was 43.9 µg/L (Gürbay et al., 2012), in other studies Ni was quantified at average levels that ranged between 1.2 µg/L (Casey and Neville, 1987; Cardoso et al., 2014) and 6.6 µg/L (Hassan, 2009). Another reported average concentration of Ni in breast milk was 5.8 µg/L (Almeida et al., 2008).

A scenario on the potential contribution of human milk was evaluated. A mean consumption of human milk of 800 mL per day and a maximum of 1 200 mL per day was considered representative for a breast-fed infant of three months and 6.1 kg b.w. (EFSA CONTAM Panel, 2011). Considering the highest reported average concentration of Ni in human milk (43.9 µg/L, from Gürbay et al., 2012), the mean dietary exposure for an infant of 6.1 kg exclusively fed with human milk would be 5.8 µg/kg b.w. per day, and for the same infant with high consumption would be 8.6 µg/kg b.w. per day. Looking at the exposure estimates in Table 9, lower or similar exposure to Ni is expected in breastfed infants as compared to non-breastfeeding infants.

Seven dietary surveys were available for 'Toddlers'. This age class showed the highest exposure to Ni together with 'Other children'. The mean dietary exposure to Ni ranged from 5.3 µg/kg b.w. per day to 13.1 µg/kg b.w. per day (minimum LB and maximum UB across European dietary surveys, respectively). The 95th percentile dietary exposure estimates ranged from a minimum LB of 8.7 µg/kg b.w. per day to a maximum UB of 20.1 µg/kg b.w. per day. For the mean dietary exposure the two surveys with the highest estimates (9.6–11.9 µg/kg b.w. per day and 11.0–13.1 µg/kg b.w. per day, LB-UB) corresponds to surveys with 36 and 17 subjects, and therefore the results should be carefully interpreted. Detailed exposure by dietary survey and age class is shown in Appendix C2.

In 'Toddlers', three food groups were the main contributors to the exposure to Ni across the different dietary surveys, 'Grain and grain-based products' (range 9.9–34.1 %, median 20.5 %), 'Milk and dairy products (range 6.0–25.0 %, median 11.4 %), and 'Vegetable and vegetable products (including fungi)' (range 4.6–12.7 %, median 8.3 %). None of these food groups possesses relatively high levels of Ni. Their role in the dietary exposure to Ni relates either to the fact that they comprise a wide and heterogeneous variety of foods or due to their high consumption in this age class (e.g. 'Milk and dairy products').

Other food groups were important contributors in specific surveys, as was the case of 'Sugar and confectionery' that peaked at 16.9 % and 19.8 % of the total contribution in two dietary surveys. A similar situation was observed for 'Non-alcoholic beverages (excepting milk based beverages)' which represented 41.8 % of the total dietary exposure to Ni in one survey due to the high consumption of cocoa beverages (Figure 8).



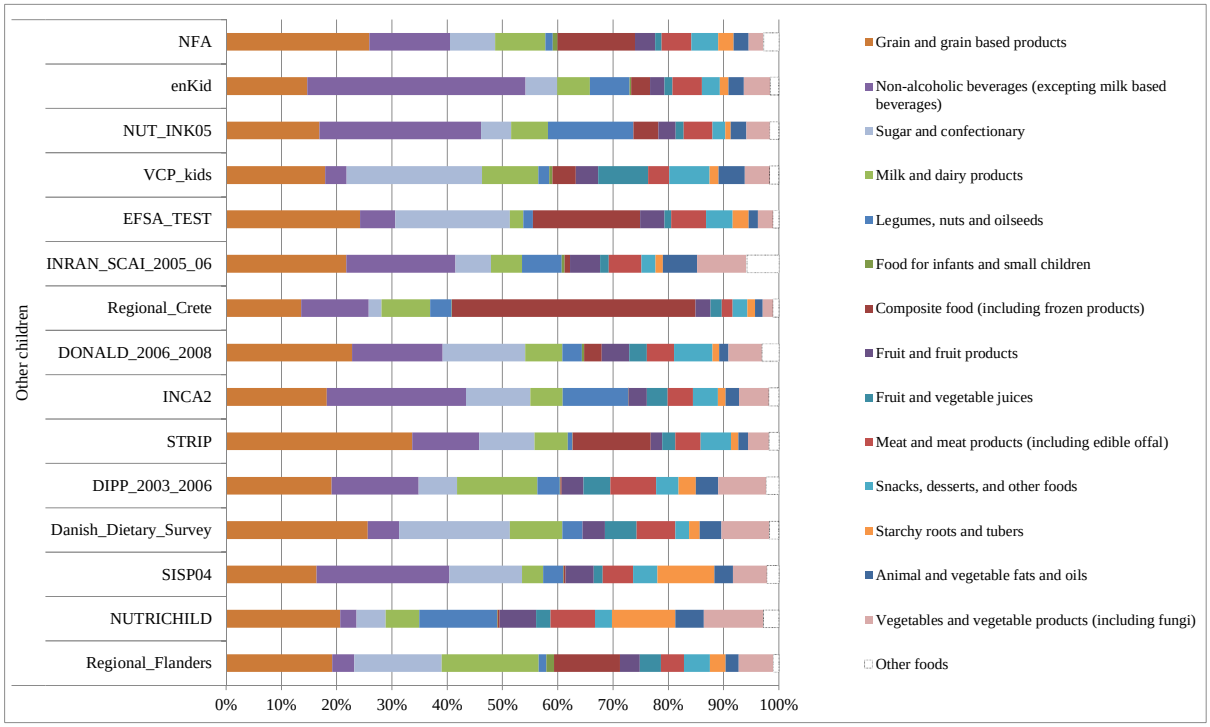
Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix C1 for more details)

Figure 8: Main food groups contributing (%) to the chronic dietary exposure to nickel for the age classes 'Infants' and 'Toddlers'

6.2.2.2. Other children

A total of 15 dietary surveys were available to evaluate the chronic dietary exposure to Ni in the age class 'Other children'. As commented above, this age class showed the highest exposure together with 'Toddlers'. The mean dietary exposure ranged from 4.9 µg/kg b.w. per day to 9.9 µg/kg b.w. per day (minimum LB and maximum UB, respectively). The 95th percentile dietary exposure estimates ranged from a minimum LB of 9.1 µg/kg b.w. per day to a maximum UB of 18.2 µg/kg b.w. per day.

The main contributor was the food group 'Grain and grain-based products' with a median contribution to the total exposure to Ni of 19.1 % across the dietary surveys (range 13.5–33.7 %). 'Non-alcoholic beverages (except milk-based beverages)' (range 3.0–39.3 %, median 14.4 %) had an important role in the dietary exposure in this age class, in particular in the dietary surveys with the highest estimates. The high presence of Ni in cocoa beverages and their relatively high consumption in certain countries explain the high contribution of this food group. Other important contributors were 'Milk and dairy products' and 'Sugar and confectionery', especially the latter with a median contribution of 10.7 % of the total, and above 10 % in eight of the dietary surveys. The contribution of 'Sugar and confectionery' is clearly driven by the high levels of Ni reported for chocolate-based products (see Appendix B).



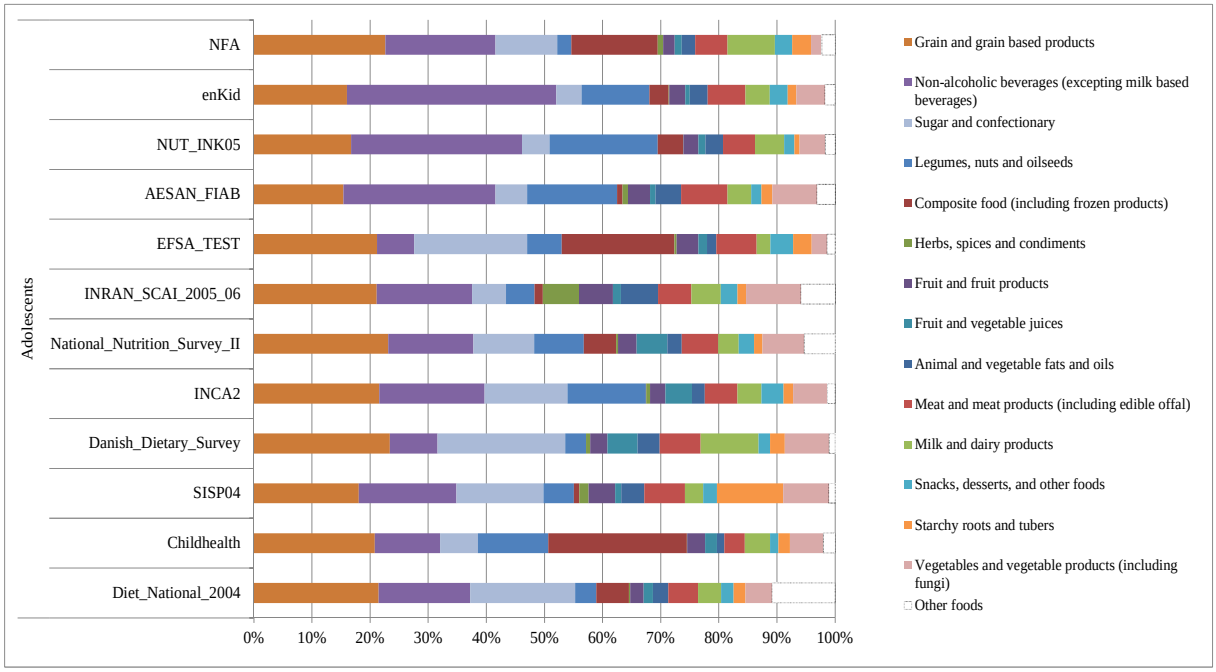
Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix C1 for more details).

Figure 9: Main food groups contributing (%) to the chronic dietary exposure to nickel for the age class 'Other children'

6.2.2.3. Adolescents

A total of 12 dietary surveys were available to estimate the chronic exposure to Ni in 'Adolescents'. The minimum value for the mean dietary exposure at the LB was 2.7 µg/kg b.w. per day, while the maximum estimated value at the UB was 5.9 µg/kg b.w. per day. For the 95th percentile dietary exposure the values ranged between 5.6 µg/kg b.w. per day (minimum LB) and 12.3 µg/kg b.w. per day (maximum UB).

As observed in the age class 'Other children', the main contributors to the dietary exposure to Ni were 'Grain and grain-based products' with a median of 21.2 % across the dietary surveys (range 15.4–23.4 %), 'Non-alcoholic beverages (except milk-based beverages)' (range 6.4–36.0 %, median 16.6 %) and 'Sugar and confectionery' (range 4.4–22.0 %, median 10.6 %). 'Cocoa beverages' and 'chocolate-based products' were the main foods responsible of the high contribution of the latter two categories. 'Legumes, nuts and oilseeds' became one of the main contributors to the dietary exposure to Ni (range 2.5–18.7 %, median = 6.7 %).



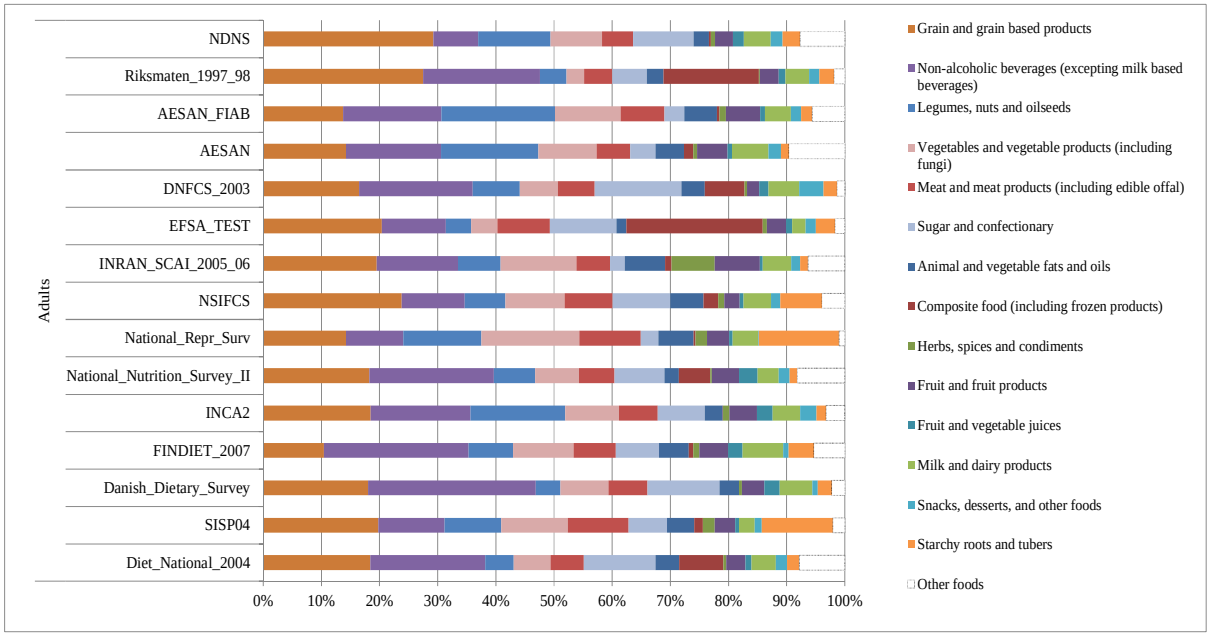
Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix C1 for more details).

Figure 10: Main food groups contributing (%) to the chronic dietary exposure to nickel for the age class 'Adolescents'

6.2.2.3. Adults

The adult population was represented by 15 dietary surveys from 14 different countries. The mean dietary exposure to Ni in the European adult population varied between 2.2 µg/kg b.w. per day and 3.6 µg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure ranged from 3.7 µg/kg b.w. per day (minimum LB) and 6.9 µg/kg b.w. per day (maximum UB).

In this age class two food groups were the main contributors to the dietary exposure to Ni, both with similar median contributions. 'Grain and grain based products' contributed the most (range 10.4–29.3 %, median = 18.4 %) followed by 'Non-alcoholic beverages' (range 7.7–28.8 %, median = 16.9 %). Unlike what was observed for 'Adolescents' and 'Other children', coffee beverages were overall the main contributor in the food group 'Non-alcoholic beverages' rather than cocoa beverages. 'Vegetables and vegetable products (including fungi)' were also important contributors (range 3.0–16.9 %, median = 9.3 %) together with 'Legumes, nuts and oilseeds' which contributions to the dietary exposure to Ni were higher than in the young population (range 4.4–19.6 %, median = 7.7 %). The food group 'Sugar and confectionery' (range 2.5–14.9 %, median = 8.1 %) was less relevant than in other age classes, although their role was still important.



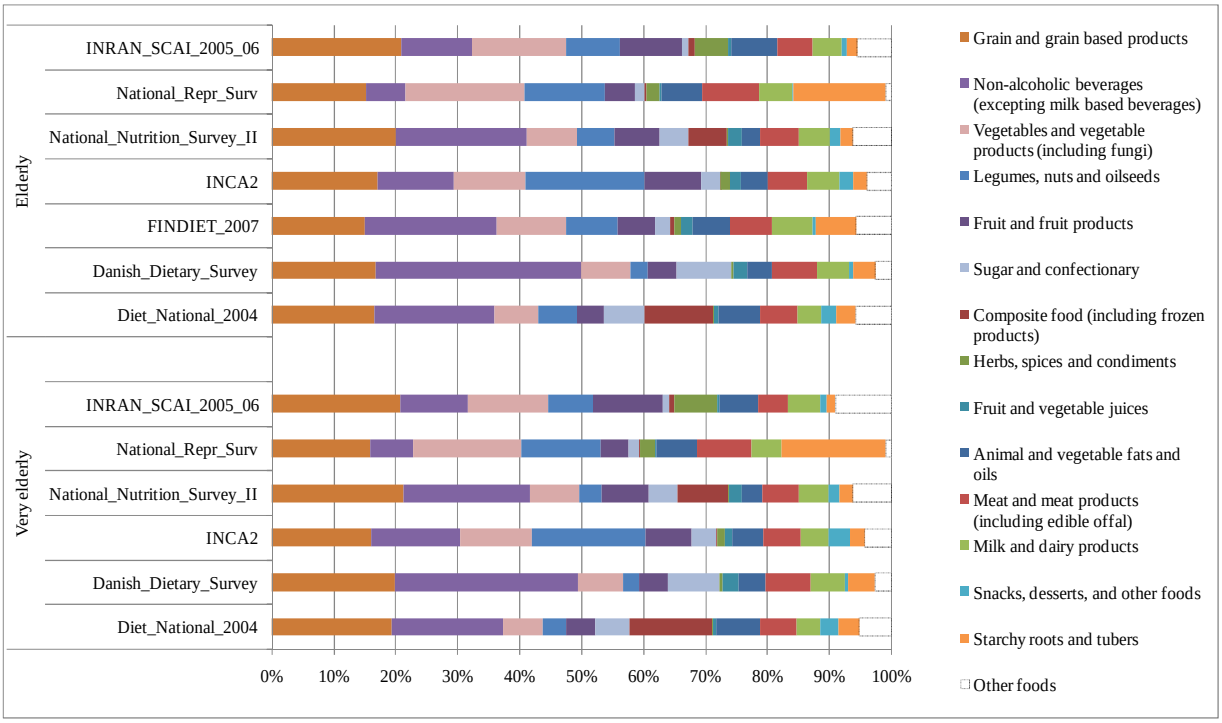
Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix C1 for more details).

Figure 11: Main food groups contributing (%) to the chronic dietary exposure to nickel for the age class 'Adults'

6.2.2.4. Elderly and very elderly

A total of seven and six dietary surveys across Europe were available for the age classes 'Elderly' and 'Very elderly', respectively. For the 'Elderly' population the mean dietary exposure to Ni ranged between 2.0 µg/kg b.w. per day and 3.2 µg/kg b.w. per day (minimum LB and maximum UB). The 95th percentile dietary exposure ranged from 3.6 µg/kg b.w. per day (minimum LB) to 5.8 µg/kg b.w. per day (maximum UB). Similar values were obtained for the 'Very elderly' population. Mean dietary exposure varied between 2.2 µg/kg b.w. per day and 3.2 µg/kg b.w. per day (minimum LB and maximum UB), while the 95th percentile dietary exposure ranged between 4.0 µg/kg b.w. per day (minimum LB) and 5.7 µg/kg b.w. per day (maximum UB).

As usually observed, in both age classes the contribution of the different food groups to the total exposure to Ni was very similar. As reported for the adult population, 'Grain and grain-based products' and 'Non-alcoholic beverages' were those food categories that contributed the most to the dietary exposure to Ni. The median contribution of 'Grain and grain-based products' across dietary surveys was 16.8 % (range 15.0–20.8 %) and 19.4 % (range 15.9–21.2 %) for the elderly and very elderly population, respectively. For 'Non-alcoholic beverages' the median contribution was higher in the elderly population (range 6.3–33.2 %, median 18.9 %) as compared to very elderly (range 6.9–29.5 %, median = 16.0 %). As observed in the adult population, coffee beverages were the main contributors within this food group. The contribution of 'Vegetables and vegetable products (including fungi)' in the elderly and very elderly populations were slightly higher than that estimated in the adult population, in particular in the elderly (range 6.9–19.2 %, median = 11.4 %). The contribution of 'Legumes, nuts and oilseeds' to the dietary exposure was higher in the elderly age class (range 2.9–19.0 %, median = 8.3 %) as compared to the very elderly age class (range 2.6–18.2 %, median = 5.5 %). As observed in the adult population the consumption of chocolate and chocolate-based products seems to be lower than in the young population what originated a drastic decrease in the contribution of the food group 'Sugar and confectionery' in these age classes. Median contribution of 3.8 % and 4.7 % of the total dietary exposure to Ni across dietary surveys were estimated in the elderly and elderly age classes, respectively.



Data are presented by individual dietary surveys across Europe using LB estimations. The names on the left refer to the names of the different surveys (see Appendix C1 for more details).

Figure 12: Main food groups contributing (%) to the chronic dietary exposure to nickel for the age classes 'Elderly' and 'Very elderly'

6.2.2.5. Conclusions

Overall, the main contributors to the dietary exposure to Ni across the different dietary surveys and age classes were 'Grain and grain-based products', 'Non-alcoholic beverages (except milk-based beverages)', 'Sugar and confectionery', 'Legumes, nuts and oilseeds', and 'Vegetables and vegetable products (including fungi)'.

The wide consumption of 'Grain and grain-based products' led this group to be a main contributor to the exposure in all age classes. The consumption of chocolate-based products and cocoa beverages and, their high levels of Ni converted the food groups 'Sugar and confectionery' and 'Non-alcoholic beverages (except milk-based beverages)' in relevant contributors to the exposure in the young population (toddlers, other children and adolescents). Although comparison with previous studies on dietary exposure to Ni is difficult due to the use of diverse food codification, the relevant role of chocolate and chocolate-products in the dietary exposure to Ni has been already reported (Arnich et al., 2012). In the adult population, 'Non-alcoholic beverages (except milk-based beverages)' were one of the most important contributors due to the consumption of coffee rather than cocoa beverages. Other food groups that were relevant contributors to the dietary exposure to Ni were 'Vegetables and vegetable products (including fungi)' and 'Legumes, nuts and oilseeds'. The influence of the first group on the exposure was most probably due to the large amount of foods included in this group. In the case of 'Legumes, nuts and oilseeds' the high levels reported for certain sub-groups such as dried beans and oilseeds, among others, was determinant in their role on the dietary exposure to Ni.

Regarding the food group 'Legumes, nuts and oilseeds', it is important to highlight that in the dietary surveys the distinction between raw and cooked/consumed food is not always clear. Following a conservative approach, when in the consumption database the food was mentioned without further details (e.g. white beans), the amounts reported were considered as raw when linked to the occurrence

data. The undetailed consumption data on 'Legumes, nuts and oilseeds' represents around 25 % of the data reported for this food group and, therefore, some overestimation of the dietary exposure to Ni and the contribution of this food group may not be discarded.

The average contribution of the different food groups in the highly exposed population (those above 90th percentile) was also assessed. Overall, the same groups that were the main contributors in the whole population were identified for the highly exposed population.

The average contribution of 'Drinking water' to the total exposure to Ni was very small across dietary surveys and age classes. At the LB scenario the contribution ranged from 0.0005 % to 1.1 %, while at the UB scenario the contribution was only slightly higher (0.0005–1.7 %). Minor contributions of drinking water to the total daily intake of Ni are usually reported in the literature (See Section 6.1).

6.2.3. Dietary exposure for specific groups

Certain foods such as nuts and beans, both with high levels of Ni, are important sources of proteins for vegetarians. Therefore, this specific group of population could be expected to have higher exposure to Ni than the general population. Unfortunately, the Comprehensive Database contains only very limited data on food consumption of people who declared they were vegetarian at the time of the survey. Considering the surveys with at least 15 adult vegetarians, the available data were grouped in five dietary surveys (Table 10). The low number of adult vegetarians included in the database makes it difficult to carry out an accurate comparison with the general population. In general, both the average and the highly exposed vegetarian population seem to have slightly higher dietary exposure to Ni than the general population. Although the differences between vegetarians and the general population are very small at the most represented dietary survey (DE/2), higher differences are observed in the second most represented dietary survey (UK) (see Table 10). However, in order to make a more appropriate estimation of the dietary exposure to Ni in the vegetarian population more consumption data for this specific group are needed.

Table 10: Comparison of the dietary exposure to nickel (µg/kg b.w. per day) between adult vegetarians and total adult population

Country	Dietary survey	N Veget.	N All	µg/kg b.w. per day			
				Mean exposure		95th percentile exposure	
				Veget.	All	Veget.	All
Lower-bound							
Finland	FI/2	39	1 575	2.5	2.4	[(a)]-	4.5
France	FR	15	2 276	3.5	2.8	[(a)]-	5.1
Germany	DE/2	237	10 419	3.0	2.6	5.8	5.2
Sweden	SE/1	18	1 210	3.4	2.8	[(a)]-	4.9
United Kingdom	UK	77	1 724	2.8	2.2	5.6	4.1
Upper-bound							
Finland	FI/2	39	1 575	3.1	3.0	[(a)]-	5.4
France	FR	15	2 276	4.3	3.4	[(a)]-	6.1
Germany	DE/2	237	10 419	3.7	3.4	7.1	6.9
Sweden	SE/1	18	1 210	4.0	3.5	[(a)]	5.8
United Kingdom	UK	77	1 724	3.3	2.8	6.4	4.9

All: total adult population; b.w.: body weight; N: number of subjects in the dietary surveys; Veget.: adult vegetarians.
(a): The 95th percentile estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.

6.3. Acute dietary exposure to nickel

Acute exposure estimates were calculated for 32 different dietary surveys carried out in 22 different European countries. As described for the estimation of the chronic exposure (see Section 6.2.2), the

available foods were grouped at FoodEx level 1 to show their contributions to the total exposure to Ni (Appendix C). Foods codified under several food categories were grouped under only one food category. This was the case for tea, coffee and cocoa (described as 'Vegetables and vegetable products' but also as 'Non-alcoholic beverages'); they were all grouped as 'Non-alcoholic beverages' when describing their contribution to the dietary exposure to Ni.

Acute dietary exposure was estimated for each reporting day by multiplying the total consumption amount for each food by an occurrence level randomly drawn among the individual results available for that food (under the UB scenario), and finally divided by the individual's b.w.. This process was iterated 100 times for each reporting day since selecting a higher number of iterations did not substantially affect the reported exposure levels at the higher percentiles. For each age class within each survey, the mean, the 95th percentile of exposure, the percentage of days with an exposure level higher than the health reference value, as well as the percentage of individuals with at least one day of exposure higher than the health reference value was characterised. For each of these endpoints, the 95 % confidence interval was defined as the 2.5th and 97.5th percentiles obtained from the 100 iterations. The food groups contributing the most to the total exposure were also identified.

6.3.1. Mean and high acute dietary exposure assessment

As observed for chronic exposure to Ni, the highest levels for acute exposure were observed in 'Toddlers' and 'Other children'. Mean and 95th percentile of acute exposure across dietary surveys and age classes are reported in detail in Appendix C3.

Mean dietary acute exposure in the young population ('Infants', 'Toddlers', 'Other children' and 'Adolescents') ranged from 3.4 (95 % CI = 3.1–3.7) µg/kg b.w. in one survey for 'Adolescents' to 14.3 (95 % CI = 13.2–15.5) µg/kg b.w.in one survey for 'Toddlers'. The 95th percentile ranged 8.6 (95 % CI = 8.0–9.1) µg/kg b.w.in one survey for 'Adolescents' to 35.0 (95 % CI = 26.8–47.2) µg/kg b.w. in one survey for 'Toddlers'. As mentioned above, the acute exposure to Ni in the adult population ('Adults', 'Elderly' and 'Very elderly') was lower than that observed in the young population. Mean dietary acute exposure ranged from 2.5 (95 % CI = 2.2–2.9) µg/kg b.w. in one survey for 'Elderly' to 4.9 (95 % CI = 4.6–5.5) µg/kg b.w. in one survey for 'Adults'. The 95th percentile ranged from 5.5 (95 % CI = 5.1–6.0) µg/kg b.w. in one survey for 'Elderly' to 11.8 (95 % CI = 10.6–13.8) µg/kg b.w. in one survey for 'Adults'. Average acute exposure estimations did not differ much from those calculated for the chronic exposure. This can be explained by the fact that Ni is present in many different foods which are regularly consumed.

The estimated acute exposure levels (minimum and maximum UB through the dietary surveys) are summarised in Table 11.

Table 11: Range of acute exposure assessment (minimum and maximum UB) to Ni across European dietary surveys. In brackets the 95 % confidence interval are reported.

Age class	Mean acute dietary exposure[(a)] (µg/kg b.w. per day)		95th percentile acute dietary exposure[(a)] (µg/kg b.w. per day)	
	Min	Max	Min	Max
Infants	5.6 (5.4-6.0)	6.4 (5.0-7.7)	15.1 (14.3-15.9)	- (b)
Toddlers	7.5 (7.0-8.1)	14.3 (13.2-15.5)	16.6 (15.0-18.8)	35.0 (26.8-47.2)
Other children	6.0 (5.5-6.8)	10.8 (98-12.6)	15.5 (13.5-18.2)	29.7 (27.8-31.8)
Adolescents	3.4 (3.1-3.7)	7.2 (6.7-7.8)	8.6 (8.0-9.1)	16.1 (15.0-17.7)
Adults	2.7 (2.6-2.9)	5.1 (4.8-5.7)	6.4 (6.2-6.6)	11.8 (10.6-13.8)
Elderly	2.5 (2.2-2.9)	4.0 (3.5-5.2)	5.5 (5.1-6.0)	9.1 (7.9-10.7)
Very elderly	2.7 (2.4-3.1)	4.0 (3.2-6.6)	6.5 (5.9-7.3)	8.7 (6.6-11.3)

b.w.: body weight.
(a): Estimates were rounded up to one decimal place.
(b): One of the surveys contains less than 60 consuming days and the 95th percentile estimates obtained in this dietary survey/age class may not be statistically robust (EFSA, 2011b). These estimates were not included in this table.

6.3.2. Food contribution to acute dietary exposure to nickel

The average contribution of different foods to the acute dietary exposure to Ni was estimated by age class across the available dietary surveys (Appendix C4). Overall, the main contributors were the same as described for the chronic exposure: 'Grain and grain based products', 'Vegetables and vegetable products (including fungi)' and 'Non-alcoholic beverages (except milk-based beverages)'. The relative contribution of groups such as 'Sugar and confectionery' and 'Legumes, nuts and oilseeds' was lower than in the dietary chronic exposure. Other important contributors to the acute exposure were 'Fruit and fruit products' and 'Milk and dairy products'. The heterogeneity of food groups such as 'Vegetables and vegetable products (including fungi)', 'Fruit and fruit products' or 'Grain and grain based products' made them to play a more relevant role in the acute exposure as compared to chronic exposure. Other factors such as a low number of occurrence data for particular foodstuffs together with a relatively wide distribution of these occurrence data may also have an influence on the contribution to the acute exposure of these food groups. In some cases, the fact that acute exposure was calculated under the UB scenario could have influenced in the higher contribution of certain food groups (e.g. 'Fruit and fruit products').

6.4. Non-dietary exposure

6.4.1. Occupational exposure

Occupational exposure to different Ni compounds was estimated in the EU RAR (2008) based on literature data, measured data from occupational monitoring in the EU and results from exposure models. Exposure scenarios were developed to cover both the production of the various compounds and their industrial applications. The EU RAR (2008) concluded that occupational exposure to aerosols may often involve many different substances (metals and non-metals) acting in concert, and Ni-bearing aerosols may contain various chemical species of Ni. To give some examples, for the refining of metallic Ni, typical inhalation exposure levels for metallic Ni and Ni-soluble species of 0.004 and 0.0064 mg/m^3 were estimated, respectively, for full day shifts (6–8 hours), 200 days/year. Typical exposure estimates to Ni-soluble species for the production and processing of Ni sulphate ranged from 0.004 mg/m^3 (production of catalysts or production of Ni compounds/salts) to 0.07 mg/m^3 (for Ni sulphate production and purification) for full day shifts (6–8 hours/day), 200 days/year. Assuming a complete respiratory absorption of the soluble Ni fraction, the CONTAM Panel estimated daily doses ranging from 0.2 to 3.9 µg/kg b.w. per day from the occupational exposure scenarios reported above, considering a 70 kg individual with a 10 m^3 inhaled air during the 8-hour shift. The EU RAR (2008) estimated also the dermal exposure during production and processing of metallic Ni and Ni compounds, which for soluble Ni compounds ranged from 0.04 to more than 18 mg total

Ni/day for metallic Ni, and from 0.04 to 1.2 mg total Ni/day for Ni sulphate. Due to the uncertainty on the dermal absorption of different Ni species, the CONTAM Panel did not estimate systemic doses from dermal exposure to Ni.

6.4.1.1. Nickel in tobacco and cigarettes

Ni can migrate from soil into tobacco plants and accumulate in the leaves. Its average concentrations in cigarette, pipe, and cigar tobacco from various geographical areas were found to vary from < 1 up to 5.5 µg/g; however, Ni content in tobacco is characterized by a remarkable variability (< 2–400 µg Ni/g, from the analysis of 12 US cigarette brands) reflecting the agronomic practices and environmental conditions of growing tobacco plants (Chiba and Masironi, 1992; Iskander et al., 1986; Smith et al., 1997; Stojanović et al., 2004).

Notwithstanding the variability of the observations, up to approximately 10–20 % of the Ni content in cigarette tobacco may possibly be released in mainstream smoke in an unidentified chemical form (IARC, 1990; Torjussen et al., 2003; ATSDR, 2005). For instance, Chiba and Masironi (1992) reported that the average Ni levels in mainstream smoke were found to be 0.0726 and 0.0785 µg/cigarette at average Ni contents of respectively 0.64 and 1.15 µg/g tobacco (in general, there is 0.7–0.9 g tobacco per cigarette); however, in mainstream smoke quite higher Ni levels (up to 0.58 µg/cigarette) were also reported (Health Canada, 1994; Smith et al., 1997). According to Health Canada (1994), Ni levels in sidestream smoke can also be as high as 0.53 µg/cigarette.

Cigarette smoke is a complex aerosol consisting of a vapour phase and a particulate phase: some experimental evidence suggests that Ni may be approximately equally distributed between the two phases (Smith et al., 1997). A mean Ni concentration of 0.03 µg/g was reported in smoke condensate collected from different US brands of cigarettes whereas most of the tobacco Ni was found to be present in the ash (Smith et al., 1997; Torjussen et al., 2003). On the assumption that a cigarette can contain Ni at an average 1–3 µg level, and that 10–20 % of Ni is released from the cigarette into the mainstream smoke, it was estimated that 2–12 µg of Ni could be inhaled for each pack of cigarettes smoked. In the EU RAR (2008), considering the most recent reviews on Ni concentrations in mainstream smoke (Smith et el., 1997; Torjussen et al., 2003), the indicative median and 95th percentile values of 0.0165 and 0.364 µg/cigarette smoke, respectively, were used for the assessment of smokers' exposure to Ni in typical and reasonable worst case (RWC) exposure scenarios. Assuming a complete absorption of the Ni present in the mainstream smoke, a heavy smoker consuming 30 cigarettes per day would be approximately exposed to a systemic dose ranging from 7 to 160 ng Ni/kg b.w. per day through cigarette smoke. This contribution is negligible or minor when compared to the dietary exposure levels estimated in this opinion.

In conclusion, both for smokers and non-smokers not-occupationally exposed to Ni (see Section 1.2.2 for Ni levels in ambient air), exposure by inhalation may be expected to represent a negligible or minor addition to the daily exposure via the diet.

6.4.2. Other exposures

Exposure to Ni via the environment was estimated in the EU RAR (2008). As discussed in Section 1.3, at the regional level the dietary exposure was by far the most important pathway accounting for > 95 % of the total exposure. In some hypothetical scenarios however, the exposure by inhalation was predicted to significantly contribute to the overall internal exposure, e.g. for local communities living in the proximities of Ni refining plants (exposure by inhalation contributing to the overall exposure up to 73 % in adults and 65 % in children) or stainless steel manufacturing plants (exposure by inhalation contributing to the overall exposure up to 39 % in adults and 43 % in children). Other pathways of exposure, e.g. soil ingestion in children, were estimated to have a low contribution both in regional and local scenarios.

7. Hazard identification and characterisation

7.1. Toxicokinetics

Several previous evaluations provide information on the toxicokinetics of Ni (US EPA, 1986; WHO, 2000; ATSDR, 2005; EFSA, 2005; EU RAR, 2008). The sections below summarise this information while presenting recent additional data in more detail.

7.1.1. Absorption

7.1.1.1. Rats

In laboratory animals Ni is rapidly but poorly absorbed following ingestion, as suggested by the low urinary excretion observed in different studies.

Ho and Furst (1973) exposed female F344 rats to 4, 16 or 64 mg Ni/kg b.w. by gavage (as ^{63}NiCl) and observed a 3–6 % excretion in the urine within 48 hours from administration, regardless of the administered dose.

Ishimatsu et al. (1995) showed that the GI absorption of Ni correlates with the water solubility of the administered Ni-containing substance. Wistar rats were administered a single dose of 10 mg Ni as metallic Ni, (green or black) Ni oxide, Ni subsulphide, Ni sulphide, Ni sulphate, Ni chloride or Ni nitrate. The substances were administered via gavage using a 5 % starch saline solution as vehicle. The absorption correlated and increased with the solubility of the Ni compound, amounting to 0.01 % for Ni subsulphide, 0.09 % for metallic Ni, 0.04 % for black Ni oxide, 0.47 % for Ni subsulphide, 9.8 % for Ni chloride, 11.12 % for Ni sulphate, and 33.3 % for Ni nitrate.

Hayman et al. (1984 - quoted in the EU RAR, 2008) described that upon oral exposure of rats to insoluble particles of a Ni alloy (particle size range of 4–6 µm) peak Ni concentrations in blood were detected about 6 hours upon dosing indicating that fine Ni particles or Ni ions released from the particles can be absorbed from the gastrointestinal tract.

7.1.1.2. Dogs

Ambrose et al. (1976) exposed Beagle dogs for two years to dietary concentrations of 100, 1 000 and 2 500 mg Ni/kg (as $NiSO_4 \cdot 6\ H_2O$, see Section 7.2.2 for the description of the study). After 12 and 24 months, collection of excreta was carried out for a consecutive week. Ni excretion was observed mainly in faeces, with 1–2 % of urinary excretion and low accumulation observed in different tissues.

7.1.1.3. Humans

In humans it was shown that the absorption of Ni is dependent on fasting state. Following oral intake via drinking water oral bioavailability of Ni is as high as about 27 % whereas absorption of Ni upon intake with food amounts to only 1 % (TERA, 1999; ATSDR, 2005).

Solomons et al. (1982) studied the oral absorption of Ni (5 mg Ni per person, as $NiSO_4 \cdot 6\ H_2O$) in adult healthy volunteers (males and females), exposed either via an aqueous solution under fasting conditions or via different beverages (tea, coffee, orange juice, whole cowmilk and a soft drink) or via two meals (one traditional Guatemalan meal including black beans, corn tortillas and coffee, and an American style breakfast including scrambled eggs, bacon, white bread, margarine and coffee). An additional experiment to study the absorption of background levels of Ni present in the Guatemalan traditional meal was included. The plasma levels of the subjects exposed via water, beverages and meals were compared to those of a group of fasted subjects not exposed to Ni. The plasma Ni levels were monitored every hour up to four hours following the exposure. The plasma levels increased significantly when Ni was given in drinking water to fasted subjects. The absorption of Ni from the administered meals showed a considerably lower absorption, with plasma levels not statistically significantly different from those in non-exposed fasted subjects. When given via a soft drink to fasted

subjects, the absorption was similar to that observed with drinking water, whereas a lower increase in plasma levels was observed following administration in whole milk, coffee, tea, or orange juice. Finally, the absorption of Ni via drinking water was also studied in the presence of disodium ethylenediaminetetraacetic acid (Na_2 EDTA) to the diet decreased the plasma Ni levels below those observed in non-exposed subjects under fasting conditions, showing a lower bioavailability of Ni complexes in comparison to free Ni.

Sunderman et al. (1989) studied the absorption of Ni in 10 human volunteers (six males and four females, ages 22–55 years). In a first experiment, study individuals were given 12–50 µg Ni/kg b.w. (as Ni sulphate) via drinking water after a 12 hour fasting period. Fasting was continued for three hours following the exposure. Following the administration of to 50 µg Ni/kg b.w. via drinking water, the first study subject experienced homonymous hemianopsia, which was suspected to be associated to the treatment. The subject was withdrawn from the study and replaced by a man of equal age. The dosage was reduced to 12–18 µg Ni/kg b.w. in the rest of the study. In a second experiment, all study subjects were exposed to the same Ni doses as in the first experiment via a standard American breakfast (including scrambled eggs, bacon, bread, margarine, jam and coffee) following a 12 hour fasting period. Blood was collected 24 hours and 1 hour before treatment and from 1 to 72 hours post-treatment. The total volume of urine (from 3 to 96 hours after treatment) and faeces (from 48 hours pre-treatment to 96 hours post-treatment) was collected for each subject. Average peak Ni concentrations in serum and urine following exposure via drinking water were 33 and 22 fold higher than respective concentrations following exposure via food. The authors calculated a mean absorption of 27 ± 17 % of the administered Ni dose for administration via drinking water, versus a respective absorption of 0.7 ± 0.4 % for administration via food.

Patriarca et al. (1997) reported, based on faecal excretion measurements, that in four fasted human volunteers 9–40 % of ingested labelled Ni (10 µg ^{62}Ni/kg b.w. in drinking water) was absorbed. The authors noted that a higher GI absorption was observed in one of the subjects, a young vegetarian woman, and speculated that it could be related to an inadequate iron dietary intake.

Nielsen et al. (1999) also studied the absorption and retention of Ni from drinking water in volunteers with or without fasting. In the first study eight non-allergic male volunteers were fasted overnight and then given Ni in drinking water at 12 µg Ni/kg b.w. and, at different time intervals, standardized 1 400 kJ portions of scrambled eggs. When Ni was ingested in water 30 minutes or one hour prior to the meal, peak Ni 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 Ni-containing water and scrambled eggs. In the latter case, a smaller, delayed peak occurred three hours after the meal. Median urinary Ni excretion half-times varied between 19.9 and 26.7 hours. The amount of Ni excreted in the three days after dosing corresponded to 2.5 % of the Ni ingested when it was mixed into the scrambled eggs. When the interval between the water and the meal increased increasing amounts of Ni were excreted, with 25.8 % of the administered dose being excreted when the eggs were served four hours prior to drinking of the Ni containing drinking water. In a second experiment, a stable Ni isotope, ^{61}Ni, was given in drinking water to 20 Ni-sensitized women and 20 age-matched controls. The course of Ni 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 than observed for the male volunteers in the first study. The authors indicated that a sex-related difference in gastric emptying rates may play a role and that food intake and gastric emptying are of substantial significance for the bioavailability of Ni from aqueous solutions.

7.1.1.4. Cellular uptake

TERA (1999) described that Ni can enter cells by three different mechanisms: uptake via metal ion transport systems, diffusion of lipophilic Ni compounds through the membrane, and phagocytosis. The cellular uptake of soluble and insoluble Ni compounds are different as insoluble Ni compounds enter the cell via phagocytosis, while soluble Ni compounds enter the cell via ion transport systems or through membrane diffusion.

7.1.2. Distribution

In rats and mice, upon oral dosing with various soluble Ni compounds, Ni was found predominantly in the kidneys (see e.g. Whanger, 1973; Ambrose et al., 1976; Oskarsson and Tjalve, 1979; Dieter et al., 1988; Ishimatsu et al., 1995). Substantial levels of Ni were also found in the liver, heart, lung, and fat (Schroeder et al., 1964; Whanger, 1973; Ambrose et al., 1976; Jasim and Tjalve, 1986b; Dieter et al., 1988) as well as in the peripheral nerve tissues and in the brain (Jasim and Tjalve 1986a; Borg and Tjalve, 1989).

7.1.2.1. Mice

In studies with mice, Ni was shown to cross the placenta, resulting in increased levels in the fetuses when given during gestation (Schroeder et al., 1964; Jasim and Tjalve, 1986a).

Radike et al. (2002) studied the tissue distribution and accumulation of Ni and other metals upon dosing a mixture of arsenic (18 mg/L), cadmium (6 mg/L), chromium (150 mg/L), Ni (150 mg/L) and vanadium (45 mg/L) via oral administration in drinking water to female B6C3F1 mice. In a second experiment female B6C3F1 mice were administered a composite sample from seven manufactured gas plant waste sites through feed. The manufactured gas plant waste mixture (MGP) included the same metals quantified at the following concentrations in feed: 47 mg As/kg, 26 mg Cd/kg, 1 105 mg Cr/kg, 1 412 mg Ni/kg, 2 376 mg Pb/kg, and 1 105 mg V/kg. In both experiments, tissues analysed included small intestine, kidneys, pancreas and femur. According to the authors, following the administration of the metal mixture via drinking water, the levels of metals and their distribution in different tissues were similar to the relative levels and distribution of the metals administered individually via drinking water. The highest metal levels were measured in the small intestine and kidneys of mice receiving the metal mixture in water. A similar tissue distribution was observed in the feed experiment with the MGP mixture, but the levels of the metals in mice receiving the MGP mixture were much lower than those in mice in mice receiving the metal mixture in water.

7.1.2.2. Rats

Ambrose et al. (1976) performed a 2-year study in rats and measured Ni levels in various tissues including bone, liver, kidneys and fat, concluding that there are no important storage sites for Ni. The study also reported a difference in bone levels between female (0.53 mg/kg) and male (< 0.096 mg/kg) rats.

Phatak and Padwardhan (1950, quoted from WHO/IPCS, 1991) fed rats with metallic Ni at concentrations of 250, 500, or 1 000 mg/kg in the diet for two months and reported that appreciable quantities of Ni from the Ni-containing diets were retained. The offspring of dams fed metallic Ni at concentrations of 500, or 1 000 mg/kg in the diet showed whole-body levels of 12–17 or 22–30 mg/kg b.w., respectively.

Szakmary et al. (1995) performed a study on the levels of Ni in maternal and fetal blood in pregnant rats unexposed or given a single dose of 5.4, 11.3 or 22.6 mg Ni/kg b.w. as Ni chloride on gestation day 19. The results revealed that at higher doses Ni concentrations in maternal and fetal blood reached a plateau whereas in amniotic fluid they were similar at all dose levels.

Cempel and Kanicka (2002) reported on the tissue distribution of Ni after oral administration of 300 and 1 200 mg/L Ni(II)chloride in drinking water to male Wistar rats for 90 days. Levels of Ni were analyzed in the liver, kidney, lung, spleen, brain, and serum by electrothermal atomic absorption spectrophotometry. The results indicate that exposed rats drank less Ni solutions than the volume of water drunk by controls. In comparison to the control animals, a very high increase in Ni levels was found in the kidney and then lung and serum of all exposed rats. In the liver, spleen, and brain the metal accumulation was lower. The increase in tissue levels of Ni was directly proportional to the Ni intake.

Li et al. (2010) studied the effects of cadmium on the absorption, distribution and excretion of Ni in rats upon dosing ^{63}Ni-$NiCl_2$ as a radiotracer in the presence or absence of $CdCl_2$ through intraperitoneal (i.p.) injection. The time–concentration curves in the blood were fitted with a two-compartment model. In the absence of co-administration of $CdCl_2$ the peak time was reported to be to 0.31 hours, whereas upon co-administration with $CdCl_2$ the value was 5.5 hours. The levels of Ni were higher at three hours and lower (close to zero) at 24 hours in all organs of interest, except kidneys. When dosed together with $CdCl_2$ there was still residual Ni(II) at 72 hours post-injection. The authors concluded that Cd(II) did affect the total Ni(II) excretion 24 hours post-injection, and that cadmium has a competitive effect on the absorption of Ni and an inhibitory effect on its elimination.

Hou et al. (2011) studied the characteristics of placental transfer and tissue concentrations of Ni in late gestational rats and fetuses by quantifying its distributions in placenta, maternal and fetal organs and tissues during the 24 hours period after a single dose of ^{63}Ni administered by i.p. injection on gestational day 20. Peak ^{63}Ni radioactivity was detected in maternal blood at 0.5 hours, in placenta and in fetal membranes, fetal blood, fetal heart, maternal kidney, lung, stomach, liver and brain at three hours, in fetal kidney, stomach, liver and brain at nine hours, and in fetal lung and amniotic fluid at 24 hours. The highest ^{63}Ni radioactivity was detected in the fetal membranes and placenta. The ^{63}Ni radioactivity in fetal blood was higher than that in maternal blood from three to 24 hours. The fetal liver, heart, stomach and brain exhibited higher ^{63}Ni radioactivity than the corresponding maternal organs from six to 24 hours. The level of ^{63}Ni in fetal lung and amniotic fluid increased throughout the study period. The authors concluded that these observations corroborate previous finding that Ni is actively transferred across the blood-placental barrier into the fetus, and that the placenta does not protect the fetus from Ni exposure. The authors also indicate that the fact that Ni concentrations are higher in most fetal organs and tissues than in corresponding maternal organs and tissues in late gestation indicates that, unlike the dam, fetuses lack effective means for getting rid of excessive Ni due to its confined environment and relatively weak kidney functions. The authors indicated that consequently, the fetuses are particularly vulnerable to the damaging effects of Ni.

7.1.2.3. Rabbits

Kalafova et al. (2012) studied the effect of dietary Ni and a combination of Ni and zinc (Zn) on the accumulation of lead (Pb), cadmium (Cd), Ni and Zn in muscles, liver and kidneys of rabbits. Female rabbits (*Oryctolagus cuniculus*) were fed a diet containing 17.5 g $NiCl_2$ per 100 kg feed, 35.0 g $NiCl_2$ per 100 kg feed, 17.5 g $NiCl_2$ plus 30 g $ZnCl_2$ per 100 kg feed, or 35.0 g $NiCl_2$ and 30 g $ZnCl_2$ per 100 kg feed for 90 days. A group fed diet without added Ni or Zn served as control. Ni exposure caused a significant increase in Cd concentration in the kidneys of the rabbits especially in the group fed 17.5 g $NiCl_2$ plus 30 g $ZnCl_2$ per 100 kg feed. In the liver an insignificant decrease of Cd concentration was found. Zn addition in the amount of 30 g to the diet caused an increase of Cd levels in the kidney as well as in the liver. The authors concluded that dietary inclusion of Ni and Zn caused specific interactions among the observed metals.

7.1.2.4. Humans

Tipton and Cook (1963) studied the presence of Ni and other metals in several tissues from autopsies of individuals non-occupationally exposed to Ni. Ni was found with high frequency in all tissues analysed, with the highest concentrations measured in the adrenal glands, colon, and skin (median levels amounting to 0.046, 0.084 and 0.33 µg Ni/g wet weight, respectively). The exact route of exposure of these individuals remained unknown.

Rezuke et al. (1987) analysed Ni levels in an autopsy study of ten individuals (six males and four females). Only one of the subjects, who worked for several years as a machinist, had a professional history with potential exposure to Ni or Ni alloys, while the other nine were non-occupationally exposed. Four of the study individuals were reported to be cigarette smokers, and another two heavy cigarette smokers. The reported mean levels from the ten subjects (in µg/kg dry weight) amounted – in decreasing order - to 174 + 94 in the lungs (the level detected in the subject occupationally exposed was excluded as an outlier), 141 + 83 in the thyroid, 132 + 84 for the adrenals, 62 + 43 in the kidneys,

54 + 40 in the heart, 50 + 31 in the liver, 44 + 16 in whole brain, 37 + 31 in spleen and 34 + 25 in pancreas. Thus, the highest concentrations of Ni were found in the lung and in the thyroid and adrenal glands (about 20–25 µg/kg wet weight) with most other organs (e.g. kidney, liver, brain) containing about 8–10 µg/kg wet weight (Rezuke et al., 1987). The body burden of Ni in adult humans was estimated to average about 0.5 mg per 70 kg (Heseker, 2000).

Maximum serum levels of Ni are observed between 1.5 and three hours after ingestion (Christensen and Lagesson, 1981; Sunderman et al., 1989; Patriarca et al., 1997; ATSDR, 2005).

Templeton et al. (1994) and Sunderman (1993) reported Ni concentrations in serum and urine from healthy persons without occupational exposure to Ni. Ni concentrations in serum/plasma and urine were in the range of 0.14–0.65 µg/L and 0.9–4.1 µg/L, respectively. For whole blood, values were to 0.34–1.4 µg/L.

7.1.2.5. Protein binding

There are some indications that when Ni reaches the systemic circulation it can bind to serum proteins, in particular to albumin.

Sarkar (1984) demonstrated that in human serum Ni is bound to proteins including albumin, and α2-macroglobulin or to L-histidine (Sarkar, 1984; Sunderman et al., 1986). The principal binding site is the histidine residue at the third amino acid position from the amino terminus in albumin from humans as well as that from rats and bovines (Hendel and Sunderman, 1972). Sarkar (1984) suggested that Ni is subjected to a ligand exchange equilibrium, and it is removed from albumin via L-histidine forming a ternary albumin-nickel-L-histidine complex, followed by formation of a low molecular weight binary L-histidine-nickel complex which can cross biological membranes. Glennon and Sarkar (1982) identified a specific Ni-binding site in human albumin, involving both the α-amino nitrogen atom of the N-terminal aspartic acid residue and the imidazole nitrogen atom of the histidine residue, as well as the involvement of the two peptide nitrogen atoms.

In rats, similar to human, the third amino acid position from the amino terminus in albumin is a prefered binding site for Ni (Hendel and Sunderman, 1972).

Dog albumin does not have a specific Ni-binding site (Glennon and Sarkar, 1982). In dogs most of the Ni (> 85 %) is not bound to proteins and as a result the relevance of studies in dogs for human risk assessment is unclear (ATSDR, 2005).

7.1.3. Excretion

7.1.3.1. Rats

Ho and Furst (1973) reported that in rats 94–97 % of the Ni administered orally was excreted via faeces and 3–6 % via urine, within a day.

Dostal et al. (1989) observed a dose-dependent increase in milk Ni concentrations four hours after subcutaneous injection of 0, 10, 50, or 100 µmol $NiCl_2$/kg b.w. (corresponding to 0.59, 2.9 or 5.9 mg Ni/kg b.w.) in lactating rats, giving milk/plasma Ni ratios of 0.02. Ni levels in milk increased until 12 hours and remained elevated at 24 hours. Repeated dosing for four days at 2.9 or 5.9 mg Ni/kg b.w. per day led to higher milk/plasma Ni ratios of 0.10 (Dostal et al., 1989).

7.1.3.2. Dogs

Ambrose et al. (1976) reported that in dogs that were given Ni sulphate in the diet for two years, only 1–3 % of the ingested Ni was excreted in urine.

7.1.3.3. Humans

In humans, Ni that is absorbed is excreted in the urine whereas Ni that is not absorbed is excreted via faeces (Torjussen and Andersen, 1979; Hassler et al., 1983; Elias et al., 1989; Ghezzi et al., 1989; Sunderman et al., 1989; Angerer and Lehnert, 1990; Patriarca et al., 1997; ATSDR, 2005).

Sunderman et al. (1989) administered 10 human volunteers with 12, 18 or 50 µg Ni/kg b.w. (as $NiSO_4$) via drinking water or food. Four days after the dosage, 26 ± 14 % of the dose of Ni administered in drinking water was excreted in urine and 76 ± 19 % in faeces. When administered in food, excretion via the faeces was 102 % ± 8 % and via urine 2 %, reflecting the lower bioavailability of Ni when dosed in food than when dosed via drinking water. The elimination half-time for absorbed Ni was reported to be 28 ± 9 hours.

Patriarca et al. (1997) administered two male and two female volunteers with 10 µg Ni/kg b.w. in drinking water and fecal and urinary excretion were studied for five consecutive days following administration. Fecal and urinary mean excretions at the end of the study period amounted to 66.9 ± 4.9 % and 22.1 ± 7.8 % of the administered dose, respectively. Five days after the administration, 51–82 % of the absorbed dose was excreted in urine, indicating retention in the body (11.0 ± 3.0 % of the administered dose).

Ni has been detected in human milk at concentrations ranging from 0.79 to 43.9 µg/L, as discussed in Section 4.1.2, indicating that Ni excretion can occur to some extent via this pathway.

7.1.4. Conclusions

All together the studies available on the absorption, distribution and excretion characteristics of Ni indicate that following ingestion, Ni bioavailability depends on the solubility of the administered Ni compound, the vehicle of administration and the fasting state of the subject. In human volunteers, the bioavailability of ingested Ni ranged from levels as low as 1 % up to 40 %. In particular a lower absorption was observed when exposure occurred in the presence of food or under non-fasted state, than when Ni was dosed in drinking water in the absence of food, or under a fasted state. The absorbed Ni can bind to serum proteins and widely distribute in the organism. Ni is actively transferred across the blood-placental barrier into the fetus that may be particularly sensitive towards the adverse effects of Ni because it lacks effective means for getting rid of excessive Ni. Absorbed Ni is excreted mainly via the urine and, to a lower extent in breast milk. An estimated elimination half life of 28 ± 9 hours was calculated in human volunteers.

7.1.5. Physiologically-based kinetic models

Sunderman et al. (1989) described a PBK model developed for oral exposure to Ni and based on two studies in eight human volunteers, in which levels of Ni in serum and faecal excretion were determined for 2 days before and 4 days after administration of Ni sulphate at dose levels of 12, 18 or 50 µg Ni/kg b.w. in water or in food to same subjects. The model was adapted from a preliminary multicompartmental model developed for rabbits and rats, and was limited to the prediction of the serum levels and urinary excretion levels following oral exposure to Ni, and did not include the prediction of Ni levels in other compartments. The adapted model included two inputs of Ni, the single oral dose administered through water or food, and the baseline dietary ingestion of Ni. The following human kinetic parameters were estimated for each subject of the two studies: a first order rate constant of intestinal absorption of Ni from the oral dose, a pseudo-zero order rate constant for fractional absorption of dietary Ni (to account for the baseline ingestion of Ni from the diet), a first order rate constant for urinary elimination, two first order rate constants for the transfer of Ni between serum and different compartments, and the mass fraction of Ni absorbed from the oral dose. The derived mean kinetic parameters were used in the PBK model. The only estimated kinetic parameter that appeared significantly different between exposure in water and food was the fraction of the dose that was absorbed, reflecting the experimental findings reported in Section 7.1.3. The adapted model was shown to adequately predict serum Ni levels. The model was not validated on an independent dataset.

7.2. Toxicity in experimental animals

7.2.1. Acute toxicity

Single-dose oral lethality studies indicate that soluble Ni compounds are more toxic than less-soluble or insoluble Ni compounds; the Ni(II) ion bioavailability being important in determining toxicity (see Table D1 in Appendix D). Ni sulphate (LD_{50}: 39–190 mg Ni/kg b.w.), Ni chloride (LD_{50}: 43–130 mg Ni/kg b.w.), Ni nitrate (> 404 mg Ni/kg b.w.) or Ni acetate (LD_{50}: 116–325 mg Ni/kg b.w.) are acutely toxic to rats whereas less soluble compounds are not acutely toxic to rats, with LD_{50} > 2 000 mg Ni/kg b.w. (Ni oxide, dihydroxide, trioxide, sulphide, subsulphide) or even higher, ranging from 8 796 to > 11 000 mg/kg b.w. for Ni oxide black or Ni oxide green (Haro et al., 1968; Itskova et al., 1969; Smyth et al., 1969; Kosova, 1979; FDRL, 1983a-h; Mastromatteo, 1986; ATSDR, 1985; Henderson et al., 2012).

Single oral administration to Wistar male rats of Ni sulphate through drinking water led to an increase of hepatic lipid peroxidation and to a decrease of antioxidant enzyme activities (Das and Dasgupta, 2002).

Non-specific effects such as hypoactivity and piloerection were observed in rats treated with Ni acetate tetrahydrate, Ni chloride hexahydrate or Ni sulphate hexahydrate. At high doses red intestines were reported.

7.2.2. Repeat dose toxicity

A table summarising the repeated toxicity studies is provided in Annex E.

The doses of Ni salts were converted to Ni doses since all studied salts are soluble, so free Ni^{2+} is the species to which animals were exposed and the anion is considered not to contribute to toxicity.

Repeated dose toxicity studies in rats or mice by the oral route (gavage, drinking water or dietary) have shown that soluble Ni compounds like acetate, chloride or sulphate induce mainly non-specific indications of toxicity such as decreases in b.w., feed or water consumption. In addition reduced survival was also often observed (see Table 11).

Decreases in liver weight were generally observed in rats or mice after oral exposure to Ni chloride or Ni sulphate. In the study of Gathwan et al. (2013), male mice exposed by gavage for 40 days to Ni chloride, in addition to decrease liver weight, hepatocyte degeneration, nuclear pycnosis, cellular swelling and congestion of blood vessels, cellular hypertrophy, increases in apoptosis and severity of necrosis were observed. The LOAEL for hepatotoxicity in this study was 8.2 mg Ni chloride/kg b.w. per day (2 mg Ni/kg b.w. per day) and the NOAEL was 2 mg Ni chloride/kg b.w. per day (0.5 mg Ni/kg b.w. per day). On the contrary, increased liver weight was observed at 45 mg Ni/kg b.w. per day in a 2-year dog study with diet Ni sulphate hexahydrate (Ambrose et al., 1976). In the study of Weber and Reid (1969) effects were observed on liver enzyme activities in mice after four weeks dietary exposure to ≥ 1 100 mg Ni acetate/kg food (≥ 200 mg Ni/kg b.w. per day). Disturbance of marker liver enzymes (alkaline phosphatase - AP, alanine transferase - ALT) following Ni treatment were observed in rats treated with 800 mg Ni sulphate hexahydrate (72 mg Ni/kg b.w. per day) for 8 weeks (Sidhu et al., 2005). The authors concluded that this finding may be the consequence of alterations in the levels of essential trace elements as a result of hepatic injury.

The kidney was the major organ of Ni accumulation (Whanger, 1973; Dieter et al., 1988; Obone et al., 1999). Decreases or increases in kidney weights were observed in several oral studies in rats or mice (Ambrose et al., 1976; Weischer et al., 1980, Obone et al., 1999). In addition, increased urinary albumin (indicator of diminished kidney function) and mild tubular nephrosis was observed in some studies in rats (Dieter et al., 1988; Vyskocil et al., 1994).

High doses of Ni can be irritating to the gastrointestinal tract, although acclimation to high levels of dietary Ni can occur (Ambrose et al., 1976; American Biogenetics Corporation, 1988). The more reliable studies are described hereunder.

Adult male Sprague Dawley rats were given 0, 0.02, 0.05 and 0.1 % Ni sulphate hexahydrate (corresponding to 0, 44.7, 111.75 and 223.5 mg Ni/L and to 0, 4, 10 and 20 mg Ni/kg b.w. per day) in their drinking water for 13 weeks. Slight decreases in b.w. were noted at the high dose. Changes in several organ weights were also noted. Decreases in both absolute and relative liver weights were observed at the two highest doses. Decreases in absolute weight of testes and heart were observed in treated animals and increases in absolute weight of kidneys, brain and spleen at high dose. There were also increases in relative spleen weight in all treated groups, in relative kidney weights at low and high dose, relative brain weight at high dose, absolute lung weights at low and high dose and relative lung weights at high dose. Total plasma proteins were decreased at the two highest dose and plasma albumin and globulins as well as plasma glutamic pyruvic transaminase activity at high dose. Lymphocyte subpopulations (T and B cells) were induced at lower dose levels but suppressed at the highest dose. A significant decrease in urine volume and an increase in blood urea nitrogen (BUN) were observed at the highest dose. Biochemical analysis of bronchoalveolar lavage fluid and lung tissue showed some lung damages (AP activity was decreased in lung tissue at high dose, decrease AP activity in bronchoalveolar lavage fluid in treated animals, and increase proteins in BALF at the two highest doses). No damage to the testes was observed. No gross or microscopic changes were seen in any of the tissues examined. The NOAEL was 4 mg Ni/kg b.w. per day (Obone et al., 1999).

Sprague Dawley rats were exposed for 91 days by oral gavage to Ni chloride hexahydrate at doses of 0, 5, 35 and 100 mg Ni/kg b.w. per day. Clinical signs of toxicity were observed at high dose. There was a dose-related increase in mortality (0, 2, 14 and 60/60 animals, respectively). Mortality at high dose and in 3/6 males and 3/8 females at the mid dose was attributed to treatment. Lower b.w. and food consumption were noted at the two highest doses. At the interim sacrifice, significant increases in WBC were seen at low and mid doses (not measured at high dose due to the decreased survival in the treatment group) as well as dose-related increases in platelet count in females, increases in differential count in neutrophils and decreases in lymphocytes at medium dose in females. There was also a dose-related decrease in glucose at the mid dose. Decreases in kidney, liver, spleen, brain and heart weights were observed in males at mid dose and decreases in kidney weight in females at the mid dose. Gastrointestinal tract (discoloured contents, distension, stomach discoloration, ulceration and smooth mucosa) and lung abnormalities (pneumonitis in 6/19 males and 9/17 females in medium dose) were observed in treated animals. Macroscopic ulcerative gastritis and enteritis was observed at high dose. No NOAEL was identified in this study. The LOAEL was 5 mg Ni/kg b.w. per day (American Biogenics Corporation, 1988).

Female B6C3F1 mice were administered 0, 1 000, 5 000 or 10 000 mg Ni sulphate/L (corresponding to 0, 33, 167 and 334 mg Ni/kg b.w. per day) orally via drinking water for 180 days. B.w. was decreased at the high dose (26 %). Decreases in absolute liver weights were noted in treated animals as well as dose-related reductions in thymus weight. The primary toxic effects of Ni sulphate were expressed in the myeloid system. There were dose-related decreases in bone marrow cellularity, in granulocyte macrophage and in pluripotent stem-cell proliferative responses. In the spleen, there was a decrease in extramedullary hematopoiesis and a reduction in the number of splenic follicles at high dose. The thymic atrophy was associated with a decrease in size of the lymphocyte-rich, thymic cortex. There were treatment-related increases in nephrosis at the mid and high doses. Effects on immune function were also noted with a dose-related reduction in spleen lymphoproliferative responses to the B-cell mitogen LPS (maximum decrease of 50 % at high dose). No NOAEL was identified in this study, the LOAEL was 33 mg Ni/kg b.w. per day (Dieter et al., 1988).

In a 90-day range-finding study, Ni sulphate hexahydrate was administered daily by oral gavage to F344 rats at levels of 0, 50, 75, 100, 125 and 150 mg $NiSO_4 \cdot 6\ H_2O$/kg b.w. per day (corresponding to 0, 11, 17, 22 28 and 33 mg Ni/kg b.w. per day). B.w. gain was reduced in an exposure-related manner in all treated groups. Males exhibited a significant reduction in b.w. gain within the first four weeks of

treatment at the two highest doses. Exposures of males in these two groups were subsequently reduced to 30 and 15 mg $NiSO_4 \cdot 6\ H_2O$/kg b.w. per day (corresponding to 7 and 3 mg Ni/kg b.w. per day), respectively, to ensure survival of the animals for the duration of the study. Following the reduction in exposure levels, b.w. gains were nearly comparable to the control group. Decreases in b.w. were observed at doses ≥ 50 mg $NiSO_4 \cdot 6\ H_2O$/kg b.w. per day. Histopathological analysis showed no treatment-related effects. The NOAEL was 30 mg $NiSO_4 \cdot 6\ H_2O$/kg b.w. per day, corresponding to 7 mg Ni/kg b.w. per day (Rush, 2002; SLI, 2002).

Ni sulphate hexahydrate was administered orally via the diet to Wistar rats for 2 years at 0, 100, 1 000 and 2 500 mg Ni/kg food (corresponding to 0, 5, 50 and 125 mg Ni/kg b.w. per day). Two-year survival was poor, particularly among control rats of both sexes and males in the high dose group, but there was no indication of an effect due to Ni. B.w. decrease was observed in both sexes in the high dose group, and sporadically for rats in the mid dose group. These decreases may be in part a result of lower food consumption. A tendency toward increased relative heart weight and decreased relative liver weight appeared in females at the two highest doses. Gross pathologic and histologic findings were essentially negative. This study has limitations as a limited number of necropsies could be performed due to the high mortality. The NOAEL was 100 mg Ni/kg food, corresponding to 5 mg Ni/kg b.w. per day (Ambrose et al., 1976).

Ni sulphate hexahydrate was administered orally via the diet to Beagle dogs for 2 years at 0, 100, 1 000 and 2 500 mg Ni/kg food (corresponding to 0, 1.8, 18 and 45 mg Ni/kg b.w. per day). All dogs survived the 2-year experimental period. During the first three days, all six dogs from the highest dose group vomited, usually within one hour. On the fourth day they returned to the control diet. All but one dog readjusted within three days. The one dog readjusted after parenteral feeding and intravenous fluids. At the start of the second week, five of the dogs were placed on 1 500 mg Ni/kg food and the sixth dog was included at the start of the sixth week. This level of Ni was well tolerated. At two-week intervals the diet level of Ni was raised to 1 700, 2 100 and 2 500 mg Ni/kg food, respectively, with no further evidence of emesis, salivation or gastrointestinal irritation. Decreased body weight was observed at the highest dose. There was a tendency toward lower haematocrit and haemoglobin values at the highest dose, suggestive of a simple hypochromic anaemia. Marked polyuria was noted in two dogs at the highest dose. Relative kidney and liver weights were higher at the highest dose. At the highest dose, all dogs showed lung lesions (multiple subpleural peripheral cholesterol granulomas, bronchiolectasis, emphysema and focal cholesterol pneumonia) and two dogs had granulocytic hyperplasia of the bone marrow. The NOAEL was 1 000 mg Ni/kg food (18 mg Ni/kg b.w. per day) (Ambrose et al., 1976).

Ni sulphate hexahydrate was administered daily by oral gavage to F344 rats for two years at levels of 0, 10, 30 and 50 mg $NiSO_4 \cdot 6\ H_2O$/kg b.w. per day (corresponding to 0, 2.2, 6.7 and 11.2 mg Ni/kg b.w. per day). There was no apparent treatment-related effect on mortality in treated males (60, 48, 50 and 57 % in control, low, mid and high dose, respectively). In females, there was an increasing exposure-response trend in mortality relative to the controls (23, 33, 43 and 45 % in control, low, mid and high dose, respectively). Not all mortalities were related to treatment: a higher rate of mortality was observed in treated animals during the first 24 weeks of the study that were secondary to aspiration of Ni sulphate solution. Starting during week 24 and continuing through the remainder of the study, oral exposure was delayed in the morning, in order to allow time for gastric empting to occur. The change in exposure time was effective in increasing survival. B.w. decreased in an exposure-dependent manner, with statistical significance at the two highest doses. No treatment-related effects were observed on clinical signs, hematology, biochemistry, urinalysis parameters, gross pathology or histopathology. The NOAEL was 2.2 mg Ni/kg b.w. per day (Heim et al., 2007).

Table 12: Repeat dose toxicity studies with Ni compounds

Study Doses in mg Ni/kg b.w. per day	NOAEL (mg Ni/kg b.w. per day)	LOAEL (mg Ni/kg b.w. per day)	Reference
40-day oral (gavage) M mouse Nickel chloride 0, 0.5, 2.0, 4.0 mg Ni/kg b.w. per day[(a)]	-	0.5	Gathwan et al. (2013)
13-week oral (drinking water) Male Rat Nickel sulphate hexahydrate 0, 4, 10 and 20 mg Ni/kg b.w. per day[(b)]	4	10	Obone et al. (1999)
91-day oral (gavage) Rat Nickel chloride hexahydrate 0, 5, 35 and 100 mg Ni/kg b.w. per day[(c)]	-	5	American Biogenics Corporation (1988)
90-day oral (gavage) Rat Nickel sulphate 0, 11, 17, 22, 28(7), 33(3) mg Ni/kg b.w. per day[(c)] Reduction of dose in two HD groups on day 28	7	11	Rush (2002) SLI (2002)
180-day oral (drinking water) Female Mouse Nickel sulphate 33, 167 or 334 mg Ni/kg b.w. per day[(a)]	-	33 mg Ni/kg b.w. per day[(a)]	Dieter et al. (1988)
2-year study oral (diet) Rat Nickel sulphate hexahydrate 0, 5, 50 and 125 mg Ni/kg b.w. per day[(b)]	5	50	Ambrose et al. (1976)
2-year study oral (diet) Dog Nickel sulphate hexahydrate 0, 1.8, 18 and 45 mg Ni/kg b.w. per day[(d)]	18	45	Ambrose et al. (1976)
104-week oral (gavage) Rat Nickel sulphate hexahydrate 0, 2.2, 6.7 and 11.2 mg Ni/kg b.w. per day	2.2	6.7	Heim et al. (2007)

b.w.: body weight; HD:
(a): calculated assuming the molecular weight of the hexahydrate salt (no information available in the original publication);
(b): calculated using EFSA default values (EFSA SC, 2012).
(c): doses reported in the study.

7.2.2.1. Conclusion

The major effects observed in repeated dose toxicity studies in rats were decreases in b.w., effects on organ weights (liver and kidneys), hepatotoxicity, nephrotoxicity, and irritation of gastrointestinal tract at high doses. In a 180-day study in mice, the primary toxic effects were observed in the myeloid system. The CONTAM Panel concluded that the lowest NOAEL for long-term exposure to Ni is 2.2 mg Ni/kg b.w. per day from a 2-year rat study. The N(L)OAELs identified in the most informative repeat dose studies are summarized in Table 12.

7.2.3. Developmental and reproductive toxicity

A table summarising the developmental and reproductive toxicity studies is provided in Annex F.

7.2.3.1. Reproductive toxicity

Several studies have examined the reproductive toxicity of Ni following oral exposure to rats, mice and dogs (WHO/IPCS, 1991; ATSDR, 2005). These studies have found conflicting results.

Pandey et al. (1999a) reported an accumulation of Ni in the epididymis, testes, seminal vesicles and prostate gland in male mice exposed by gavage to 5 or 10 mg Ni sulphate/kg b.w. per day (corresponding to 1.1 or 2.2 mg Ni/kg b.w. per day) (5 days/week) for 35 days. There was no change in b.w., but a decrease in weights of testes, epididymis, seminal vesicles and prostate gland was observed. The accumulation of Ni in male reproductive tissues resulted in histopathological damages in these tissues (at 2.2 mg Ni /kg b.w. per day atrophy of centrally located tubules and disturbed spermatogenesis (decrease in sperm motility and total sperm count), damages in epididymis were observed) and sperm damages. In addition, male mice from the control group and exposed to 2.2 mg Ni sulphate/kg b.w. per day for 35 days were mated with untreated females. A decrease in the fertility index was observed in the treated group. In females mated with treated males a decrease in the number of pre- and post-implantations and an increase in resorptions were observed. A decrease in weight was also observed in fetuses from dams mated with treated males. The authors concluded that the testicular and spermatotoxic changes may be responsible for observed male mediated developmental toxic effects.

Panday and Srivastava (2000) reported also dose-related decreases in weights of reproductive organs (testes, epididymis, seminal vesicles and prostate gland), in mice exposed by gavage to 20 mg Ni sulphate or Ni chloride/kg b.w. per day for 35 days. Decreases in sperm motility and count and increases in abnormal sperm were observed at 10 and 20 mg Ni sulphate or Ni chloride/kg b.w. (corresponding to 2.2/2.5 and 4.5/5 mg Ni /kg b.w.). At comparable doses, the spermatotoxic effects were of higher severity for Ni chloride than for Ni sulphate (see results summarised in Table 13). The NOAEL was 5 mg Ni sulphate or Ni chloride/kg b.w. per day (1.1/1.3 mg Ni/kg b.w. per day). The authors concluded that the abnormal and non-motile sperm may reduce the fertilizing capacity of spermatozoa and adversely affects the fertilization of ovum. The CONTAM Panel noted that in this study only a limited number of parameters have been investigated - b.w. gain, male reproductive organ weights and sperm parameters -, and that only six males were tested per group.

Table 13: Effects of Ni on sperm motility, total epididymal sperm count and sperm abnormalities in mice treated with Ni chloride or Ni sulphate for 35 days (Panday and Srivastava, 2000)

Dose mg Ni/kg b.w. per day	Motile sperm (%)		Sperm count epididymis (10^7)		Abnormal sperm (%)	
	Ni sulphate	Ni chloride	Ni sulphate	Ni chloride	Ni sulphate	Ni chloride
0	88.3	86.0	8.0	8.0	8.5	8.7
1.1°/1.3°°	85.8	85.1	8.5	8.2	17.9	18.9
2.2°/2.5°°	75.0*	65.0*	7.0	6.0*	24.4	29.1
4.5°/5.0°°	65.0*	49.1*	6.0*	5.0*	28	34.6

*: $P < 0.05$, mean of 6 mice/group; °: conversion from Ni sulphate; °°: conversion from Ni chloride.

Young male Swiss albino mice were given a daily oral dose of 0 (0.9 % NaCl) or 20 mg Ni sulphate/kg b.w. per day (corresponding to 0 or 4.5 mg Ni/kg b.w. per day) for 5 days/week for six months. There was no sign of toxicity in any of the treated animals, but after six month of exposure, mean b.w. was reduced in treated animals. The urinary excretion of protein (testosterone-dependent) was lower in treated mice compared with controls. Testicular weight and histology did not differ in the two groups. Lower weight and smaller size (diameter) of the seminal vesicles was observed in exposed males. There was also a lower secretory activity of the cells of the vesicular epithelium. Ni

accumulated in the interstitial tissue of the testes. These effects are similar to those expected when the seminal vesicle is subjected to decrease testosterone levels. The authors concluded that the decreased production of testosterone may therefore be an early effect of long-term Ni exposure (Pandey and Singh, 2001).

Sobti and Gill (1989) reported increases in sperm head abnormalities in epididymes in mice receiving a single gavage dose of 23, 28 or 43 mg Ni/kg b.w. as Ni nitrate, Ni sulphate or Ni chloride, respectively (study poorly reported).

Käkelä et al. (1999) reported reduction in the number of pregnancies when male rats were exposed via drinking water to 30 mg Ni chloride/kg b.w. per day (7.41 mg Ni/kg b.w. per day) for 28 or 42 days before copulation. The decrease in fertility was higher in rats exposed for 28 days than in rats exposed for 42 days, suggesting regeneration of damaged tissues. In the testes, Ni chloride induced shrinkage of the seminiferous tubules, which seemed to close some of the tubules. A significant decrease in basal spermatogonia was also observed in the rats exposed for 28 days but not in the rats exposed for 42 days. It is to be noted that the final b.w. of males exposed for 28 days appear to be lower than control b.w. Female-only exposure to concentrations as high as 100 mg Ni chloride/kg b.w. per day (24.7 mg Ni/kg b.w. per day) in drinking water did not adversely affect fertility in rats (Käkelä et al., 1999). Interpretation of this study is limited by the small number of animals tested (six/gender/group) and the limited reporting of the results.

Other studies have not found histological alterations in male or female reproductive tissues in rats administered up to 100 mg Ni/kg b.w. per day as Ni chloride hexahydrate for 91 days (American Biogenic Corporation, 1988), rats exposed to 20 mg Ni/kg b.w. per day as Ni sulphate in drinking water for 90 days (Obone et al. 1999), rats exposed to 2.2 mg Ni/kg b.w. per day as Ni sulphate hexahydrate administered via gavage for 18 weeks (2-GEN study, SLI, 2000b), rats exposed to 125 mg Ni/kg b.w. per day as Ni sulphate in the diet for 2 years (Ambrose et al., 1976), or dogs exposed to 45 mg Ni/kg b.w. per day as Ni sulphate in the diet for 2 years (Ambrose et al., 1976).

No alterations in sperm count, concentration, motility, or morphology were observed in the F0 or F1 rats administered 2.2 mg Ni/kg b.w. per day as Ni sulphate via gavage for 18 weeks (SLI, 2000b).

No adverse effects on fertility or on the reproductive performances were observed in a 2-GEN study in which male and female rats exposed to doses as high as 42 mg Ni/kg b.w. per day as Ni chloride hexahydrate in drinking water for 11 weeks prior to mating (RTI 1988a, b), in a one-generation study in which rats were administered 17 mg Ni/kg b.w. per day as Ni sulphate hexahydrate via gavage for two weeks prior to mating, during mating, and during gestation (SLI, 2000a), in a two-generation study involving gavage administration of up to 2.2 mg Ni/kg b.w. as Ni sulphate hexahydrate per day for 10 weeks prior to mating, during mating, gestation, and lactation (SLI, 2000b), or in a 2-litter study in which female rats were exposed to doses as high as 31.6 mg Ni/kg b.w. per day (Smith et al., 1993).

Toman et al. (2012) demonstrated the adverse effect of Ni on the mouse testis structure from three to 12 weeks of administration in feed of 10 mg $NiCl_2$/kg b.w. per day. The most vulnerable site is the seminiferous epithelium which undergoes degeneration and the germ cells desquamate from the Sertoli cells connections in the tubule lumen creating empty spaces in the epithelium and die. The interstitial tissue was also significantly affected. The changes in the testis become more visible the longer are the periods of Ni exposure. This study shows that oral administration of Ni causes serious damage to the spermatogenesis and development of the testis structure, when administered for long-term to young mice at the beginning of their sexual maturity.

7.2.3.2. Developmental toxicity

Ni crosses the placental barrier, affecting directly the developing embryo or fetus in experimental animals (Jacobsen et al., 1978; Sunderman et al., 1978; Olsen and Jonsen, 1979). Apparently, Ni can enter the embryo from day five to eight of pregnancy but not earlier (Jacobsen et al., 1978; Olsen and

Jonsen, 1979). In late gestation stages, Ni concentrations in mouse fetuses increase (Olsen and Jonsen, 1979) and can even be higher in fetal organs than in maternal ones (Jacobsen et al., 1978).

The available animal data on developmental toxicity show that the developing fetus and neonates are sensitive targets of Ni toxicity. Käkelä et al. (1999) reported decreases in the number of pups born alive, the number of pups surviving until post natal day (PND) 4 and litter size at PND 21 when male rats were exposed via drinking water to 30 mg Ni chloride/kg b.w. per day (7.41 mg Ni/kg b.w. per day) for 28 days before copulation. However, when the male rats were exposed to 30 mg Ni chloride/kg b.w. per day for 42 days, no significant alterations in pup viability or survival were observed. The pups that died during lactation were runts: the heads were disproportionately large and the posteriors of the bodies were underdeveloped and they moved slowly. Exposure of female rats to 100 mg Ni chloride/kg b.w. per day (24.7 mg Ni/kg b.w. per day) in drinking water for 14 days prior to mating, during mating, gestation, and lactation resulted in a decreased pup survival from birth to PND 4 and from PND 4 to 21. No significant alterations were observed at 30 mg Ni chloride/kg b.w. per day (7.41 mg Ni/kg b.w. per day). High pup mortality was also observed when both parents were treated with 30 mg Ni chloride/kg b.w. per day. When the females were treated with 100 mg Ni chloride/kg b.w. per day, the liver and kidneys of their pups weighed relatively less than those of the control pups. A NOAEL was not identified in this study.

An increase in spontaneous abortions was observed in female mice exposed to 160 mg Ni/kg b.w. per day as Ni chloride in drinking water on GD 2–17 (Berman and Rehnberg, 1983); no effects were observed at 80 mg Ni/kg b.w. per day. In contrast, no effects on the average number of neonates per litter were observed when mouse dams were treated by gavage on GD 8–12 with 90.6 mg Ni/kg b.w. per day as Ni chloride (a dose that resulted in a significant decrease in maternal b.w.) (Seidenberg et al., 1986). However, the multi-generation reproduction toxicity studies indicate that increased perinatal mortality is induced during the later parts of the gestation and the early postnatal periods.

Pup mortality was also observed in a multi-litter study in which rats were exposed to 0, 1.3, 6.8, or 31.6 mg Ni/kg b.w. per day as Ni chloride in drinking water for 11 weeks prior to breeding and during two successive gestation and lactation periods (Smith et al., 1993). In the first litter, the numbers of litters with dead pups at birth were 5, 5, 0 and 11 and the percentages of dead pups per litter at PND 1 were 1.7, 3.1, 0, and 13.2 % (statistically significant at the high dose only); no significant alterations were observed in the number of dead pups at PND day 21. In the second litter, the number of litters with dead pups at birth (2, 7, 6, and 10; statistically significant at high dose only), the percentages of dead pups per litter at PND 1 (1.0, 4.3, 4.6, and 8.8 %; statistically significant at all three dose levels), and the percentage of dead pups at PND 21 (12.5, 13.4, 19.4, and 29.2 %; significant at high dose only) were increased. It is not possible to know if the pre-weaning deaths are a result of an inherent defect in the pups, Ni exposure through the milk, or a change in the quality or quantity of the milk produced by the dam (Smith et al., 1993). Decreased birth weight was noted in males from the first litter at 6.8 mg Ni/kg b.w. per day (see results summarised in Table 14). The NOAEL for maternal toxicity was 1.3 mg Ni/kg b.w. per day, based on decreases in body weight gain at the next higher dose and the LOAEL for offspring toxicity is 1.3 mg Ni/kg b.w. per day.

Table 14: Reproductive outcome of female rats exposed to Ni chloride (Smith et al., 1993)

Dose mg Ni/kg b.w. per day	N	Sperm positive females	Number of viable litters	Mean pups/litter (live and dead)	Nb. Litters with dead pups at birth	Total dead pups PND1 (% dead pups/litter)	Total dead pups PND21 (% dead pups/litter)
1st breeding							
0	34[(a)]	29	25	12.9	5	5 (1.7)	38 (11.5)
1.3	34	30	25	12.2	5	9 (3.1)	32 (7.6)
6.8	34	30	24	11.7	0	0 (0)	10 (2.8)
31.6	34	32	27	13.2	11	35*** (13.2)	55 (15.0)
2nd breeding							
0	29[(b)]	28	23	10.6	2	2 (1.0)	22 (12.5)
1.3	29	28	22	12.5	7°	11** (4.3)**	33 (13.4)
6.8	30	29	24	13.3	6	16* (4.6)°	61 (19.4)
31.6	31	31	25	11.3	10**	22*** (8.8)***	69 (29.2)**

N: number of female rats; °: $0.05 < P \leq 0.10$; *: $0.03 < P \leq 0.05$; **: $0.01 < P \leq 0.03$; ***: $0.001 < P \leq 0.01$.
(a): number of females entering study,
(b): number of females bred for second time

In a three-generation study (Ambrose et al., 1976) involving exposure of rats to 0, 5, 50, or 125 mg Ni/kg b.w. per day as Ni sulphate hexahydrate (0, 250, 500 and 1 000 mg Ni/kg food) in the diet for 11 weeks prior to mating, during mating, gestation, and lactation, a dose-related increase in the number of stillborn pups was observed in F1 pups (the number of pups born dead was increased in all treated groups in F1a generation and at the two high dose in the F1b generation). A decrease in the number of fetuses/litter was observed at the high dose as well as dose-related decreases in the number of weaning fetuses/litter. At the high dose, b.w. of weanlings decreased in all generations, with recovery between weaning and subsequent mating. No macroscopic or histopathological lesions were observed in weanlings. The NOAEL for parental toxicity was 50 mg Ni/kg b.w. per day, based on a slight decrease in b.w. at the high dose, the NOAEL for reproduction was 125 mg Ni/kg b.w. per day (the highest dose tested) and the LOAEL for offspring toxicity was 5 mg Ni/kg b.w. per day.

A two-generation study was conducted by RTI (1988a, b) in which the P0 generation was exposed to Ni chloride hexahydrate in drinking water at doses of 0, 50, 250 and 500 mg/L for 11 weeks before mating and during gestation and lactation, and the F1b generation animals were mated to produce the F2 generations. The doses correspond to 0, 6.0, 25 and 42 mg Ni/kg b.w. per day for P0 and 0, 6.2, 23 and 42 mg Ni/kg b.w. per day for F1b. A reduction in live litter size was observed in the F1a, F1b, and F2a offspring of rats exposed to 42 mg Ni/kg b.w. per day. Increases in mortality were also observed in the F1b rats on PND 22 through 42; these increases were statistically significant in males at 25 and 42 mg Ni/kg b.w. per day and in females at 42 mg Ni/kg b.w. per day. No adverse developmental effects were observed in the caesarean-delivered F2b rats, suggesting that the Ni-induced decrease in live litter size occurred post-natally. No gross abnormalities were observed in the surviving offspring of rats exposed to Ni. Death of female rats from pregnancy complications at the time of delivery suggests that females are more susceptible to Ni toxicity during parturition. Although the number of deaths was not significantly above controls and not clearly dose-related (P0: 0/31 in controls, 1/31 at 6 mg/kg/day, 3/30 at 25 mg/kg b.w. per day, and 3/31 at 42 mg/kg b.w. per day; F1: 0/30 at 0 and 6.2 mg/kg b.w. per day, 3/30 at 23 mg/kg b.w. per day, and 1/30 at 42 mg/kg b.w. per day), death in dams during delivery is a relatively rare event. The results of this study (RTI, 1988a, b) are

confounded by a decrease in food and water intake observed in the exposed animals. Decreases in pup b.w. were reported in the offspring of rats exposed to 25, and 42 mg Ni/kg b.w. per day (RTI, 1988a, b). The NOAEL for parental toxicity was 25 mg/kg b.w. per day based on significant decreases in b.w. at 42 mg Ni/kg b.w. per day, the NOAEL for reproductive toxicity was 42 mg Ni/kg b.w. per day and the NOAEL for developmental toxicity was 6 mg Ni/kg b.w. per day. The authors concluded that Ni exposure interferes primarily with the normal processes associated with late gestation, parturition, lactation and/or postnatal development and that the severity of these effects shows considerable variability among individual females and their litters.

In a dose range-finding 1-generation study, significant increases in post-implantation losses (mean post-implantation loss 0.4, 2.6, 1.5, 2.3, 2.7 and 4.8 and number of litters with post-implantation loss: 2/8, 5/8, 6/8, 6/7, 7/7 and 8/8 at 0, 2.2, 4.4, 6.6, 11 and 17 mg Ni/kg b.w. per day) were observed in the offspring of rats administered ≥ 6.6 mg Ni/kg b.w. per day as Ni sulphate hexahydrate via gavage for 14 days prior to mating, during mating, and gestation (SLI, 2000a). The number of dead pups at lactation day 0 (stillbirth) was significantly increased in all exposure groups except the 11 mg Ni/kg b.w. per day group (1/128, 12/100, 10/106, 10/92, 4/89 and 23/80), and at 17 mg Ni/kg b.w. per day, a decreased mean litter size was observed. No effect on growth of surviving F1 pups during lactation and no effect on survival or growth of F1 pups from PND 22 for several weeks following weaning was observed (see Table 15). The CONTAM Panel identified a NOAEL for parental toxicity of 17 mg Ni/kg b.w. per day (the highest tested dose) and a LOAEL of 2.2 mg Ni/kg b.w. per day for offspring toxicity, based on the number of dead pups at PND 0.

Table 15: One-generation dose range-finding study in rats (SLI, 2000a)

Dose mg Ni/kg b.w. per day	0[(a)]	2.2	4.4	6.6	11	17
Mean post-implantation loss	0.4	2.6	1.5	2.3*	2.7**	4.8**
Number of litters with post-implantation loss	2/8	5/8	6/8	6/7	7/7	8/8
Number of litters with at least 3 post-implantations loss	0/8	1/8	1/8	2/7	3/7	7/8
Number of dead/live pups, day 0	1/128	12/100**	10/106**	10/92**	4/89	23/80**

b.w.: body weight; Ni: nickel.
(a): Historical control: mean: 1.5 (0.88–2.31); *: $P < 0.05$; **: $P < 0.01$.

In a 2-GEN reproduction toxicity study, Ni sulphate hexahydrate was administered by gavage to rats at levels of 0, 0.2, 0.6, 1.1 and 2.2 mg Ni/kg b.w. per day (SLI, 2000b). According to the authors, no effect on F1 or F2 pup viability and growth was observed in the offspring of rats administered up to the highest dose tested, 2.2 mg Ni/kg b.w. per day. The authors reported therefore a NOAEL for developmental toxicity of 2.2 mg/kg b.w. per day. The mean combined post-implantation/perinatal lethality until postnatal day 0 among the F1 offspring was higher at 2.2 mg Ni/kg b.w. per day, however, the difference was not statistically significant (0.9, 1.5, 1.2, 1.3 and 2.1). In the F2 offspring, the value was similar to the F2 control value. As perinatal lethality also occurs after the day of birth, the Danish EPA wanted to evaluate the whole time period from implantation to perinatal day four as a continuum. For the highest dose group, the post-implantation combined with the perinatal lethality on day four was statistically significantly increased in the F1 generation (P-value of 0.058 and p-value of 0.044 in Mann-Whitney test for the mean percentile of combined post-implantation/peri-natal lethality) (1.0, 1.2, 1.2, 1.3 and 2.3 = 7.1 %, 8.1 %, 8.7 %, 11.0 % and 15.8 %) (see Table 16). On this basis, the Danish EPA established a NOAEL of 1.1 mg/kg b.w. per day (EU RAR, 2008). Historical control group mean values for post-implantation/prenatal loss at day 0 from 8 studies ranged from 0.88 to 2.31 pups per litter. The value of 2.1 per litter for the group exposed to 2.2 mg Ni/kg b.w. per day is within this range. There was no statistically significant effect on post-implantation/peri-natal lethality in the F2 offspring.

The CONTAM Panel decided to apply a benchmark dose approach to derive a point of departure on the dose-response curve.

Table 16: Two-generation study in rats (SLI, 2000b)

Dose (mg Ni/kg b.w. per day)	0	0.2	0.6	1.1	2.2
F0/F1 generation					
Mean post-implantation loss day 0	0.9	1.5	1.2	1.3	2.1
Number of. litters with post-implantation loss (%)	13/25 (52)	18/26 (69)	15/25 (60)	19/26 (73)	19/28 (68)
Number of. litters with at least 3 post-implantation loss (%)	3/25 (12)	3/26 (12)	5/25 (20)	5/26 19)	9/28 (32)
Mean post-implantation loss + perinatal lethality day 4	1.0	1.2	1.2	1.4	2.3**
Post-implantation loss + perinatal lethality day 4 (%)	7.1	8.1	8.7	11.0	15.8*
F1/F2 generation					
Mean post-implantation loss day 0	0.9	1.9	1.3	1.3	1.2
Number of litters with post-implantation loss (%)	13/24 (54)	18/26 (69)	16/25 (64)	18/23 (78)	14/24 (58)
Number of litters with at least 3 post-implantation loss (%)	0/24 (0)	4/26 (15)	3/25 (12)	3/23 (13)	4/24 (17)

b.w.: body weight; Ni: nickel.
Historical control: mean: 1.5 (0.88-2.31), * $p< 0.05$, ** $p<0.01$. The cut-off of 3 post-implantation losses was based on the maximum value in the historical controls of 2.31.

In an evaluation of the potential effects of Ni on functional development, no effects on figure eight maze reactive locomotor activity levels were observed in the offspring of mice treated by gavage at 45.3 mg Ni/kg b.w. per day as Ni chloride (100 mg/kg b.w. per day) on GD 8–12 (Gray et al., 1986).

Saini et al. (2013) studied the effects of oral (gavage) exposure during gestation (GD6-13) of Swiss albino mice to Ni chloride hexahydrate at doses of 0, 46, 92 and 185 mg Ni/kg b.w. per day. Maternal toxicity (decrease feed consumption, water intake and b.w.) was observed at doses ≥ 92 mg Ni/kg b.w. per day and fetotoxicity (decreases in b.w.), embryotoxicity (decrease in the number of live fetuses/dam, increases in post-implantations losses and resorptions at high dose), and teratogenicity (malformations such as open eyelids, club foot, umbilical hernia, ophthalmic anomalies, hydrocephaly, reduced ossification, dose-dependent increase in skeletal anomalies) were observed at doses ≥ 92 mg/kg b.w. per day (microphthalmia already at 46 mg/kg b.w. per day). The NOAEL for maternal toxicity was 46 mg Ni/kg b.w. per day and the LOAEL for developmental toxicity was 46 mg Ni/kg b.w. per day.

Table 17: Developmental and reproductive toxicity studies with nickel compounds

Study Doses in mg Ni/kg b.w. per day	NOAEL (mg Ni/kg b.w. per day)	LOAEL (mg Ni/kg b.w. per day)	Reference
Reproductive toxicity: 1–3 generations studies			
2-GEN study oral (drinking water) Rat Nickel chloride hexahydrate 0, 6.0/6.2, 25/23 and 42/42 mg Ni/kg b.w. per day. Average exposure premating/mating period: Males 0, 4, 19 and 31 mg/kg b.w. per day[(a)] Females 0, 3, 12 and 22 mg/kg b.w. per day Exposure ranges gestation period: 5-6, 22-26, 33-44 mg/kg b.w. per day[(a)] Exposure ranges post natal period (GD20–PND21) 4-13, 12-58, 14-98 mg/kg b.w. per day[(a)]	Parental toxicity: 25 Reproduction toxicity: 42 Offspring toxicity: 6	Parental toxicity: 42 Reproduction toxicity: - Offspring toxicity: 25	RTI (1988a, b)
3-generation study oral (diet) Rat 30M + 30F/group (F0, F1b, F2b) ➔ after 11wk: 20F mated with 20M Nickel sulphate hexahydrate 0, 5, 50, 125 mg Ni/kg b.w. per day[(b)]	Parental toxicity: 50 Reproductive toxicity: 125 Offspring toxicity: -	Parental toxicity: 125 Reproductive toxicity: - Offspring toxicity: 5	Ambrose et al. (1976)
1-generation Oral (gavage) Rat Nickel sulphate hexahydrate 0, 2.2, 4.4, 6.6, 11 and 17 mg Ni/kg b.w. per day	Parental and reproductive toxicity: 17 Offspring toxicity: -	Parental and reproductive toxicity: - Offspring toxicity: 2.2	SLI (2000a)
2-GEN oral (gavage) Rat Nickel sulphate hexahydrate 0, 0.2, 0.6, 1.1 and 2.2 mg Ni/kg b.w. per day	Parental, reproductive and offspring toxicity: 2.2	Parental, reproductive and offspring toxicity: -	SLI (2000b)
2-litter study 11-week prior to mating + during 2 successive gestation + lactation periods Oral (drinking water) F Rat Nickel chloride 0, 1.3, 6.8, 31.6 mg Ni/kg b.w. per day[(a)] Mated with untreated M	Maternal toxicity: 1.3 Offspring toxicity: -	Maternal toxicity: 6.8 Offspring toxicity: 1.3	Smith et al. (1993)

Table continued overleaf.

Table 17: Developmental and reproductive toxicity studies with nickel compounds (continued)

Study Doses in mg Ni/kg b.w. per day	NOAEL (mg Ni/kg b.w. per day)	LOAEL (mg Ni/kg b.w. per day)	Reference
Oral (drinking water) Rat Nickel chloride hexahydrate F: control F: 2.47, 7.41 and 24.7 mg Ni/kg b.w. per day 14 days before mating, mating, gestation and lactation F: 7.41 mg Ni kg b.w. per day 100 days before mating, mating, gestation and lactation F: 24.7 mg Ni/kg b.w. per day + 0.3 mg/L Se 14 days before mating, mating, gestation and lactation M: 7.41 mg Ni kg b.w. per day 28 days before mating M: 7.41 mg Ni kg b.w. per day + 0.3 mg/L Se 28 days before mating M: 7.41 mg Ni kg b.w. per day 42 days before mating M + F: M: 7.41 mg Ni kg b.w. per day 28 days before mating and mating mated with F: 7.41 mg Ni kg b.w. per day 28 days before mating, mating, gestation and lactation	Reproductive toxicity: -	Reproductive toxicity: 2.47	Käkelä et al. (1999)
Reproductive organs toxicity			
2-year study oral (diet) Rat Nickel sulphate hexahydrate 0, 5, 50 and 125 mg Ni/kg b.w. per day[(b)]	Systemic toxicity: 5 Reproductive toxicity: 125	Systemic toxicity: 50 Reproductive toxicity: -	Ambrose et al. (1976)
2-year study oral (diet) Dog Nickel sulphate hexahydrate 0, 1.8, 18, 45 mg Ni/kg b.w. per day[(c)]	Systemic toxicity: 18 Reproductive toxicity: 45	Systemic toxicity: 45 Reproductive toxicity: -	Ambrose et al. (1976)
13-week oral (drinking water) M Rat Nickel sulphate 0, 4, 10 and 20 mg Ni/kg b.w. per day[(b)]	Systemic toxicity: 4 Reproductive toxicity: 20	Systemic toxicity: 10 Reproductive toxicity: -	Obone et al. (1999)

Table continued overleaf.

Table 17: Developmental and reproductive toxicity studies with nickel compounds (continued)

Study Doses in mg Ni/kg b.w. per day	NOAEL (mg Ni/kg b.w. per day)	LOAEL (mg Ni/kg b.w. per day)	Reference
91-day study oral (gavage) Rat Nickel chloride hexahydrate 0, 5, 35 or 100 mg Ni/kg b.w. per day[(a)]	Systemic toxicity: - Reproduction toxicity: 100	Systemic toxicity: 5 Reproduction toxicity: -	American Biogenic Corporation (1988)
35-day gavage (5 days/week) M mouse Nickel sulphate 0, 1.1 or 2.2 mg Ni/kg b.w. per day[(d)]	Systemic toxicity: 1.1 Reproductive toxicity: -	Systemic toxicity: 2.2 Reproductive toxicity: 1.1	Pandey et al. (1999)
35-day gavage (5d/wk) M mouse Nickel sulphate 0, 1.1, 2.2 or 4.5 mg Ni/kg b.w. per day[(d)] or Nickel chloride 0, 1.3, 2.5 or 5 mg Ni/kg b.w. per day[(d)]	Systemic toxicity: 1.1 (sulphate) or 1.3 (chloride) Reproductive toxicity: -	Systemic toxicity: 2.2 (sulphate) or 2.5 (chloride) Reproductive toxicity: 1.1 (sulphate) or 1.3 (chloride)	Panday and Srivastava (2000)
6-month gavage (5d/wk) M mouse Nickel sulphate 0 or 4.5 mg Ni/kg b.w. per day[(d)]	Systemic and reproduction toxicity: -	Systemic and reproduction toxicity: 4.5	Pandey and Singh (2001)
3-6-9- and 12-week oral (pellets) M mouse Nickel chloride 0 or 2.5 mg Ni/kg b.w. per day[(d)]	-	2.5	Toman et al. (2012)
Male mediated developmental toxicity			
35-day gavage (5 d/wk) M mouse Nickel sulphate 0 or 2.2 mg Ni/kg b.w. per day[(d)] Mated with untreated females (15 dams/dose)	-	2.2	Pandey et al. (1999)
Developmental toxicity			
GD 6-13 oral (gavage) F mouse Nickel chloride hexahydrate 0, 46, 92 or 185 mg Ni/kg b.w. per day[(a)] Sacrifice on day 18	Maternal toxicity: 46 Developmental toxicity: -	Maternal toxicity: 92 Developmental toxicity: 46	Saini et al. (2013)

2-GEN: 2-generation; b.w.: body weight; d: day; F: female; GD: gestation day; M: male; Ni: nickel; PND: post-natal day; wk: week.
(a): doses reported in the study.
(b): calculated using EFSA default values (EFSA SC, 2012).
(c): approximate estimation using allometric scaling.
(d): calculated assuming the molecular weight of the hexahydrate salt (no information available in the original publication).

7.2.3.3. Conclusions

In rat reproductive toxicity studies and repeated dose toxicity studies, oral administration of Ni compounds did not induce alterations in reproductive tissues and no adverse effects on fertility or reproductive performances were reported. The CONTAM Panel concluded that the lowest NOAEL for effects on fertility in rats is 2.2 mg Ni/kg b.w. per day in a 2-GEN study where animals were exposed by gavage to Ni sulphate hexahydrate (SLI, 2000b). However, in mice, effects on male sex organs

weights, histopathological changes in these organs, disturbed spermatogenesis, decreased sperm motility and sperm damages have been reported in studies after oral exposure to doses ≥ 2.2 mg Ni/kg b.w. per day and were responsible for a decrease in fertility. In these studies, several limitations were noted: number of animals tested, number of doses tested, and number of parameters investigated. Therefore, the CONTAM Panel considered that these studies could not be used for establishment of an RP. In a limited study in rats, a decrease of fertility was also reported when males exposed to Ni chloride hexahydrate at a dose of 7.41 mg Ni/kg b.w. per day were mated with untreated females.

For developmental toxicity, Ni crosses the placental barrier, affecting directly the developing embryo or fetus. There is consistent evidence of increased pup mortality (stillbirth or post-implantation/perinatal lethality) after exposure of rats to Ni chloride or sulphate in several reproductive toxicity studies at doses ≥ 1.3 mg/kg b.w. per day. The CONTAM Panel decided to apply a benchmark dose approach on the data from a range-finding reproductive toxicity study and a 2-GEN reproductive toxicity study to derive an RP on the dose-response curve for the incidence of litters with post-implantation loss per treatment group (see Section 7.6).

Decreases in fetuses or pups weights were observed at higher doses. In mice exposed to Ni chloride, malformations, reduced ossification and increased incidence of skeletal anomalies were observed at doses ≥ 92 mg Ni/kg b.w. per day in the presence of maternal toxicity. However, microphthalmia was observed at 46 mg Ni/kg b.w. per day in the absence of maternal toxicity. Ni is considered to be a developmental toxicant inducing fetotoxicity, embryotoxicity and teratogenicity. The N(L)OAELs identified in the most informative studies are summarised in Table 17.

7.2.4. Genotoxicity

The genotoxicity of Ni compounds has been reviewed by several organizations including IARC (1990), US EPA (1996), TERA (1999), ATSDR (2005), and EU RAR (2008). This section contains a summary of the data. The most relevant studies are described in detail.

7.2.4.1. *In vitro* studies

DNA damage

Water-soluble as well as water insoluble Ni compounds have been shown to induce DNA single strand breaks (SSBs), DNA protein crosslinks (DPCL) and oxidative base damage in mammalian test systems.

Robison and Costa (1982) showed that both $NiCl_2$ and crystalline αNiS induced DNA strand breaks as detected by alkaline sucrose gradient analysis in cultured Chinese hamster ovary (CHO) cells. These Ni compounds caused DNA strand breaks at concentrations which did not significantly affect cell proliferation.

Nackerdien et al. (1991) investigated the ability of Ni(II) ions to cause chemical changes in DNA in chromatin extracted from human cultured cells in the presence of H_2O_2. The products that were identified were typical hydroxyl radical-induced products of DNA bases. The partial inhibition of product formation by typical scanvengers of hydroxyl radicals confirmed the idea that hydroxyl radicals were involved in their formation. Ni(II) in the presence of H_2O_2 induced more base damage to DNA in chromatin than to isolated DNA. The authors hypothesize that this might be due to the ability of complexes of Ni(II) with certain peptide sequences in chromatin to generate free radicals in the presence of oxygen.

Kawanishi et al. (2001, 2002) explored the induction of oxidative damage by a variety of Ni compounds (Ni_3S_2, NiO (black), NiO (green) and $NiSO_4$) in cultured cells, RaJi and HeLa cells. Among Ni compounds only Ni_3S_2 induced DNA strand-breaks as detected by pulsed field gel electrophoresis, and increased levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) compared with control. Nitric oxide (NO) generation in phagocytic cells (RAW 264.7 cells) was induced by all Ni

compound tested. On the basis of this and previous (Inoue and Kawanishi, 1989; Kawanishi et al., 1989) studies, the authors propose two mechanisms for Ni-induced oxidative DNA damage: i) induction of indirect damage via inflammation, and ii) induction of direct oxidative damage via H_2O_2 formation as in the case of Ni_3S_2.

Patierno and Costa (1987) reported the first evidence of the enhancement of DNA protein binding by Ni(II) in intact mammalian cells. Chakrabarti et al. (2001) analysed the induction of DPCL by Ni compounds in isolated rat lymphocytes. The soluble form of Ni subsulphide induced DPCL levels significantly higher than those induced by Ni sulphate at doses where there was no reduction of cell viability. Co-incubation of Ni subsulphide with aminoacids, such as L-histidine, L-cysteine or L-aspartic acid, significantly reduced the levels of DPCLs as well as the accumulation of Ni^{2+} in lymphocytes suggesting that these aminoacids play a protective effect against genotoxicity of Ni subsulphide by reducing the cellular uptake of Ni^{2+}. *In vitro* exposure of lymphocytes to Ni subsulphide increased also the formation of ROS. Since co-incubation of Ni subsulphide with catalase, dimethylthiourea, mannitol or vitamin C significantly decreased DPCL formation the authors concluded the formation of DPCLs by Ni subsulfide is caused by the formation of ROS. The aminoacid treatment also abrogated Ni subsulphide-induced ROS formation. Deferoxamine, a specific iron chelator, prevented the formation of DPCLs suggesting that the first step in their formation is the induction of a Fenton/Haber-Weiss reaction generating hydroxyl radicals.

M'Bemba-Meka et al. (2005) analysed the induction of DNA SSBs by Ni sulphate hexahydrate ($NiSO_4 \cdot 6\ H_2O$), Ni subsulphide (Ni_3S_2) Ni oxide (NiO) and Ni carbonate hydroxide tetrahydrate (NiCH) ($2\ NiCO_3 \cdot 3\ Ni(OH)_2 \cdot 4\ H_2O$) in human whole blood lymphocytes in culture. Lymphocytes were exposed to low concentrations (0–15 μM) of the different Ni compounds for 2 hours. The capacity of induction of DNA SSBs decreased in the following order: NiCH > Ni oxide ≥ Ni subsulphide > Ni sulphate. Pre-treatment of human blood lymphocytes with ROS scavengers or GSH precursors significantly reduced DNA SSBs induced by NiCH in both chromosomal and nuclear chromatin, suggesting the involvement of oxidative stress in SSB induction.

Pre-treatment with an iron chelator prevented NiCH-induced DNA SSBs in both chromosomal and nuclear chromatin suggesting that iron-mediated oxidative stress generating hydroxyl radicals is involved in SSB induction. Simultaneous treatment with inhibitors of Ca^{2+} through plasma membranes or mobilization of Ca^{2+} from endoplasmic reticulum, or the use of a Ca^{2+} chelator significantly reduced Ni compound-induced DNA SSBs in both chromosomal and nuclear chromatin, suggesting that Ni compound-induced destabilization of calcium homeostasis may also be involved in the induction of SSBs.

Schwerdtle and Hartwig (2006) compared soluble Ni chloride and poorly soluble Ni oxide with respect to uptake, intracellular distribution and genotoxicity as detected by the comet assay in the A549 human lung cell line. Both compounds were taken up by the cells and led to elevated concentrations in the cytoplasm as compared to the nucleus with a higher fraction reaching the nucleus in the case of NiO. However, also the exposure to Ni(II) led to increased nuclear Ni content indicating that water soluble Ni compounds may also interact with nuclear molecules. Similar effects for Ni chloride and Ni oxide were observed with respect to the induction of DNA SSBs and oxidative damage as revealed by Fpg treatment. Both compounds showed the most pronounced effects after long treatment times (20–24 hours) and at cytotoxic concentrations. On the basis of these findings and previous studies the authors propose that the higher carcinogenic potential of particulate Ni compounds may be due to much longer retention times *in vivo* (and therefore persistent DNA repair inhibition) more than to different mechanisms of action at cellular level.

Caicedo et al. (2008) studied DNA damage (by the comet assay) and apoptosis induced by a variety of metal ions including Ni. In particular, Ni sulphate hexahydrate ($NiSO_4 \cdot 6\ H_2O$), Ni subsulphide (Ni_3S_2) Ni oxide (NiO) and Ni carbonate hydroxide tetrahydrate (NiCH) ($2\ NiCO_3 \cdot 3\ Ni(OH)_2 \cdot 4\ H_2O$) were tested in human (Jurkat) T-cells. Ni, together with vanadium, induced the most DNA

damage and was the most apoptotic among the metals tested, inducing > 50 % caspase-9 positive T cells at 0.05 mM.

Gene mutations

Ni compounds are inactive in almost all bacterial mutagenicity tests (Arlauskas et al., 1985; Marzin and Phi, 1985; Biggart and Costa, 1986) and are weakly mutagenic in cultured mammalian cells. The most relevant studies conducted in mammalian cells are summarized below.

A slight increase of 8-azaguanine resistance (mutations at the hypoxanthine-guanine phosphoribosyltransferase (*hprt)* gene) was detected in Chinese hamster V79 cells following treatment with Ni chloride but only at highly cytotoxic doses (Miyaki et al., 1979).

Mutagenesis of several insoluble Ni compounds -crystalline Ni sulphide, Ni subsulphide, Ni oxides- and soluble $NiCl_2$ was studied at the *hprt* gene of V79 Chinese hamster cell lines and at gpt in two transgenic derivative cell lines, G12 and G10 (Kargacin et al., 1993). A high increase in *gpt* mutation frequency was reported only in the transgenic G12 cell line and only after treatment with the insoluble Ni compounds. Vitamin E was able to suppress some of the cytotoxic and mutagenic activity of the insoluble Ni compounds supporting the hypothesis that oxidative damage may play a key role in Ni mutagenicity. The soluble $NiCl_2$ was only weakly mutagenic in G12 cell lines as well as in the other V79 cell lines. Klein et al. (1994) hypothesized that in the G12 cells, Ni mutagenesis may be related to the integration of the *gpt* gene into a heterochromatic region of the genome. Lee et al. (1995) showed that the Ni-induced inactivation of *gpt* expression (without mutagenesis or deletion of the transgene) was reversed by the demethylating agent 5-azacytidine. This finding suggests the involvement of DNA methylation in silencing *gpt* expression. This was confirmed by demonstrations of increased DNA methylation, as well as by evidence indicating condensed chromatin and heterochromatinization of the gpt integration site in 6-thioguanine-resistant cells. This paper supports the theory that Ni is a human carcinogen that can alter gene expression by enhanced DNA methylation and compaction, rather than by mutagenic mechanisms.

Mayer et al. (1998) showed increased mutation frequency by Ni subsulphide in a lacI transgenic embryonic fibroblast cell line. In about one-third of the mutants the molecular analysis did not reveal any mutation although there was phenotypic loss of the lacI function suggesting alternative mechanisms of gene silencing. Moreover, they applied the comet assay to freshly isolated mouse nasal mucosa and lung cells to investigate the induction of DNA damage in target cell of carcinogenesis. DNA SSBs were detected in a dose-dependent manner in both cell types.

There is one report of induction of high mutation rates together with chromosomal instability (Ohshima, 2003). Five out of 37 clones (13.5 %) derived from Ni sulphate-treated V79 cells showed a remarkably increased frequency of *hprt* mutations, while only one out of 37 control clones (2.7 %) showed this high mutation rate. In addition, 17 out of 37 clones (45.9 %) from Ni-treated cells showed structural chromosomal aberrations in 10 % or more of cells (up to 45.5 %), while only three out of 31 control clones (9.7 %) showed this high aberration rate. Out of 37 clones derived from Ni-treated cells, eight (21.6 %) and 11 (29.7%) clones showed an increased frequency (≥ 5 %) of aneuploid and polyploid cells, respectively, while only a few control clones showed such an increase in aneuploid and polyploid cells.

Three human lung tumour cell lines A427, HCC15 and NCI-H2009 were transfected with a mammalian expression vector containing a (CA)(13) repeat in the coding sequences of the reporter hygromycin gene and used to study whether Ni(II) may induce microsatellite mutations in human cells (Zienolddiny et al., 2000). Soluble Ni(II) induced microsatellite mutations consisting of both contraction and expansion of the CA repeat unit.

In rat kidney epithelial cells (NRK) infected with MuSVts110 retrovirus, Ni(II) induced insertion mutation at a 70 bp-long stretch of DNA (Chiocca et al., 1991).

The analysis of the type of mutations induced by Ni support that oxidative damage is involved. CHO cells cultured with Ni(II) or Ni_3S_2 showed predominantly deletion mutations (Rosetto et al., 1994). The G > T transversion, typical of oxidative damage, was found in the K-*ra*s gene (codon 12) in renal tumours induced by Ni_3S_2 alone or combined with iron powder (Higinbotham et al., 1992). The same type of mutation was detected in the *p53* gene associated with Ni-exposure related lung tumours (Harty et al., 1996)

Although there is very limited evidence of Ni mutagenicity, several reports indicate that Ni ions may be co-mutagenic.

Dubins and La Velle (1986) found that Ni ions enhance the mutagenicity of alkylating agents in a bacterial fluctuation test.

Hartwig and Beyersmann in 1989 showed the $NiCl_2$ is able to induce mutations at the *hgprt* locus as well as sister chromatid exchanges (SCEs) in V79 Chinese hamster cells and shows a pronounced comutagenic effect towards UV light. This is likely due to interference with DNA repair processes (see also below).

Chromosomal alterations

Water-soluble and poorly water-soluble Ni compounds induce SCE, chromosomal aberrations and micronuclei at high (millimolar), cytotoxic levels in different mammalian cell systems. These effects are likely due to aneugenic as well as clastogenic actions.

The ability of Ni compounds to induce chromosome aberrations was first reported by Nishimura and Umeda (1979). Since then many studies have reported the induction of chromosome aberrations, SCE, micronuclei by Ni compounds (Larramendy et al., 1981; Ohno et al., 1982; Waksvik and Boysen, 1982; Sen and Costa, 1985; Arrouijal et al., 1992). Swierenga and Basrur (1968) and Anderson and Mehandru (1985) reported the spindle-inhibiting effect of Ni compounds and suggested that aneuploidy might be induced.

Seoane and Dulout (2001) studied the aneugenic and clastogenic ability of a serie of metals including Ni chloride (II) and Ni sulphate (II) by using the kinetochore-stained micronucleus test in human diploid fibroblasts (MRC-5). Ni salts induced a weak but significant increase in micronuclei frequency. The increase in kinetochore-positive micronuclei was higher than in kinetochore-negative ones indicating aneugenic as well as clastogenic effects. An aneugenic-related effect of Ni sulphate was previously reported by Li et al. (1996) and Beron et al (1995).

Ohshima (2003) reported the induction of aneuploidy by Ni sulphate in V79 Chinese hamster cells. This effect was clearly indicated by chromosome numbers and increased frequency of kinetochore-positive micronuclei. In addition $NiSO_4$ induced abnormal chromosome segregation in anaphase/telophase cells due to asymmetric segregation. The authors hypothesize that this due to an effect of the compound on the spindle apparatus

Sen and Costa (1985) showed that exposure of Chinese hamster ovary cells to water-soluble $NiCl_2$ and to particulate crystalline NiS induced a dose-dependent increase of chromosomal aberrations. In particular the exposure to crystalline NiS particles induced a high incidence of chromatid exchanges and dicentrics and produced what the authors define an effect on the condensation state of the heterchromatic long arm of the X chromosome. The authors hypothesize that the pathway of delivery of Ni^{2+} from NiS particles may be responsible for a preferential interaction of Ni with heterochromatic long arm of the X chromosome. The selective effects of Ni on genetically inactive heterochromatin was one year later proposed by the same authors (Sen and Costa, 1986) for the increased incidence of SCEs induced by $NiCl_2$ in Chinese hamster ovary cells characterized by preferential induction of these exchanges in the heterochromatic regions of the chromosomes. In 1987 a complete analysis of the localization of chromosomal aberrations following treatment with $NiCl_2$ and crystalline NiS was

carried out in Chinese hamster ovary cells and C3H10T1/2 cells and compared with the effects of $CaCrO_4$ (Sen et al., 1987). It was shown that the chromosomal aberrations induced by Ni occurred predominantly in heterochromatic regions of the chromosomes and the specific effect of NiS on the condensation state of the heterochromatic long arm of the X-chromosome was confirmed. Conversely chromate was shown to interact with chromatin randomly. The interaction of Ni with nuclear proteins in heterochromatin was proposed as a mechanism of carcinogenesis.

Cell transformation

Water-soluble and poorly water-soluble Ni compounds induced anchorage-independent growth and morphological transformation in different cell systems.

Costa and Mollenhauer (1980) showed that the crystalline Ni sulphide and subsulphide compounds were actively phagocytozed by Syrian hamster embryo cells and Chinese hamster ovary cells whereas amorphous Ni sulphide was not taken up in significant quantities. Water soluble Ni compounds had significantly less transforming activity and DNA SSBs (as detected by alkaline sucrose gradients) induction than the crystalline Ni sulphide and subsulphide in Syrian hamster embryo cells (Costa et al., 1982) thus suggesting that the induction of DNA damage and cellular transformation by these compounds is proportional to their cellular uptake (i.e. selective phagocytosis).

Conway and Costa (1989) examined the ability of Ni compounds (NiS and $NiCl_2$) to transform to anchorage independence early passage Chinese hamster embryo (CHE) cells and identified non-random karyotypic changes in the anchorage-independent transformants. Two- to three-fold more male anchorage-independent transformants than female transformants were obtained from the Ni-treated cultures. Deletions of the long arm of the X chromosome were specifically identified in the transformants suggesting that these deletions may be associated with the transformation process in these cells.

Miura et al. (1989) compared the ability of insoluble and soluble Ni compounds to induce cell transformation in C3H/10T1/2 Cl 8 (10T1/2) mouse embryo fibroblasts. Soluble Ni sulphate and Ni chloride caused dose-dependent cytotoxicity after 48 hours treatments, but neither compound induced morphological transformation even at concentrations causing up to 94 % cytotoxicity. Conversely, insoluble Ni subsulphide, Ni monosulphide, and Ni oxide caused dose-dependent cytotoxicity and a low, dose-dependent frequency of morphological transformation. Interestingly, no induction of base substitution mutations to ouabain resistance was observed over concentration ranges that induced morphological transformation. One transformed cell line obtained following induction by Ni oxide formed tumours in nude mice.

7.2.4.2. *In vivo* studies

DNA damage

There is evidence that both soluble and insoluble Ni compounds give rise to both DNA breaks and DNA-protein crosslinks *in vivo*.

Saplakoglu et al (1997) reported the formation of DNA SSBs, as detected by the alkaline unwing assay, in rat lung and kidney after acute treatment of animals with $NiCl_2$ (44.4 mg/kg b.w.) injected subcutaneously. In the rat liver, no DNA SSBs were detected. The combined treatment with Ni and cadmium ($CdCl_2$, 4 mg/kg b.w.) reduced the number of SSBs.

Kawanishi et al. (2002) investigated the induction of oxidative DNA damage by a variety of Ni compounds in rats following intratracheal instillation. A significantly increased level of 8-OH-dG was measured by HPLC-ECD in lungs of rats treated with 1 mg of Ni compounds. The order of the increase was Ni_3S_2 > NiO (black) ≈ NiO (green) > $NiSO_4$. Treatment with 0.5 mg of Ni compounds also increased 8-OH-dG in lungs, with the exception of NiO (green) and $NiSO_4$. The authors propose that, *in vivo*, Ni compounds mostly induce indirect oxidative damage via inflammation with the

exception of Ni_3S_2 that also showed direct induction of oxidative damage via H_2O_2 formation. This double mechanism might account for its relatively high carcinogenic potential.

In the study by Danadevi et al. (2004) Swiss albino mice were administered orally (by gavage) with acute doses of 3.4, 6.8, 13.6, 27.2, 54.4 and 108.8 mg/kg b.w. of $NiCl_2$ and samples of whole blood were collected at 24, 48 and 72 hours, first week and second week post-treatment for alkaline comet assay. A significant increase in mean comet tail length indicating induction of single/double-strand breaks was observed with $NiCl_2$ at all-time intervals except in the 2nd week post treatment in comparison to controls. A gradual decrease was reported at 72 hours indicating the occurrence of repair. These data clearly indicate that $NiCl_2$ is able to induce DNA damage *in vivo*.

Gene mutations

In vivo mutation studies with Ni compounds were mostly conducted in *Drosophila melanogaster* and showed weakly positive effects.

Ni chloride was tested in the wing spot test in *Drosophila melanogaster* (Ogawa et al., 1994). Oral treatment was weakly positive with the highest effect recorded at 12 mM.

Rasmuson (1985) screened Ni salts, $NiCl_2$ and $Ni(NO_3)_2$, for mutagenicity using a sensitive somatic eye-colour test system in *Drosophila melanogaster*. Larval feeding with 0.14 mM $Ni(NO_3)_2$ and 0.21 mM $NiCl_2$ did not cause somatic mutations above the control level.

Rodríguez-Arnaiz and Ramos (1986) tested Ni sulphate in *Drosophila melanogaster* males injected i.p. for the induction of mutations in germ cells. Significant increases of sex-linked recessive lethals was observed at all concentrations tested (200, 300, 400 mg/L) while total sex-chomosome loss was only detectable at the highest concentration.

Mayer et al. (1998) investigated the genotoxic effects of Ni subsulphide *in vivo* in LacZ transgenic CD2F1 mice and in lacI transgenic F34 rats used for comet assay and mutagenicity analysis, respectively. Ni_3S_2 was administered by inhalation with an estimated total uptake of 4–13 mg Ni_3S_2/kg b.w. A significant increase of induced DNA strand breaks could be found in nasal mucosa cells of CD2F1 mice following two hours inhalation of Ni_3S_2, leaving only 40 % of the cells undamaged. *In vivo* mutagenicity data in nasal mucosa and lung tissue of mice or rats exposed as in the comet assay showed no increase of mutation frequencies compared to negative controls.

The Ni-induced oxidative stress response was investigated in testis of adult albino mice following i.p. administration of multiple sublethal doses of Ni chloride (1.25, 2.5 and 5.0 micromol/100 g b.w. per day for three or five days) (Doreswami et al, 2004). A moderate increase in lipid peroxidation was observed in testis in association with a significant increase in DNA SSBs as measured by a DNA unwinding assay and increased apoptosis at higher doses. Increased percentages of abnormal sperms were also recorded in Ni-treated males during the first three weeks. Mating of Ni treated males (2.5 micromol/100 g b.w. per day for five days for five weeks) with untreated females resulted in a significant increase in male-mediated dominant lethal-type mutations (frequency of dead implantations) during the first three weeks.

Chromosomal effects

The induction of chromosomal aberrations and micronuclei in rodents treated with different Ni compounds is not consistent across studies.

No clastogenic effects in polychromatic erythrocytes were reported by Deknudt and Leonard (1982) in male mice i.p. injected with doses of 25 mg/kg $NiCl_2$ or 56 mg/kg $Ni(NO_3)_2 \cdot 6\ H_2O$. These compounds did not increase the rate of post-implantation death but decreased significantly the rate of pregnancy as well as the amount of pre-implantation loss.

El-Habit and Abdel Moneim (2014) examined the ability of cadmium and Ni, alone or in combination, to induce genotoxicity, cytotoxicity, and oxidative stress in bone marrow cells of male mice. Each animal received the assigned dose subcutaneously (s.c.) once a day for three consecutive days. Ni chloride was used in three doses (40, 80, and 120 μmol/kg b.w./injection). A dose-related significant increase of polychromatic erythrocytes with micronuclei was observed in bone marrow cells following animal exposure to Ni as compared to control. Increased frequency of bone marrow cells with aneuploidy and chromosomal aberrations were also induced by Ni, although the effects were lower as compared to those induced by cadmium. Treatment of mice with Ni(II) and Cd(II) salts simultaneously decreased the incidence of micronucleated PCEs in bone marrow cells. Cd and Ni were found to induce also significant DNA damage in mouse bone marrow cells as assessed by the comet assay and a dose-dependent increase of oxidative stress markers (i.e. lipid peroxidation and nitric oxide) with a significant decrease of the antioxidant GSH content.

Following oral administration there are a few contrasting studies.

Sharma et al. (1987) analysed chromosomal aberrations following oral administration of $NiSO_4$, $NiNO_3$ and $NiCl_2$ to mice for 4, 8, 12 and 16 days. All the Ni compounds induced increased frequency of chromosomal aberrations as compared to control at the tested doses of 95, 73 and 72.2 mg/kg b.w. for $NiCl_2$, $NiSO_4$ and $NiNO_3$, respectively. In the same study the authors observed increased frequency of chromosome inversions by all Ni compounds in a mosquito species, *Anopheles stephensi*.

Sobti and Gill (1989) investigated the induction of micronuclei and sperm head abnormalities of a variety of Ni salts. Oral administration of $NiCl_2$ (95 mg/kg b.w.), $NiSO_4$ (73 mg/kg b.w.) and $NiNO_3$ (72.2 mg/kg b.w.) induced a statistically significant increase in the micronuclei frequency in mice. The frequency of sperm abnormalities was also increased.

Dhir et al. (1991) investigated the effects of *Phyllanthus emblica* and ascorbic acid against the clastogenicity induced by Ni in mice. Animals were treated with different doses of Ni chloride (10, 20 and 40 mg/kg b.w.) injected intraperitoneally. The animals were sacrificed 6, 12 and 24 hours after the administration of the Ni salt. Dose-related increased frequency of both chromosomal aberrations and micronuclei were reported in Ni treated mice as compared to control.

Oller and Erexson (2007) showed that the oral administration (by gavage) of Ni sulphate hexahydrate (125, 250, and 500 mg/kg b.w. per day) did not induce statistically significant increases in micronucleated polychromatic erythrocytes (PCEs) in rat bone marrow of young adult male rats of the Sprague–Dawley strain at any dose examined. This study was conducted according to OECD and EU protocol guidelines.

In conclusion, although the information is scanty, there are *in vivo* data confirming the *in vitro* clastogenicity of Ni compounds.

Table 18: *In vivo* DNA damage and chromosomal alterations induced by nickel compounds administered orally

Form of nickel	Experimental system/route of administration	Type of effect	Result	Reference
Nickel chloride $NiCl_2$ 1.5, 3.1, 6.2, 12.3, 24.6 and 49.3 mg Ni/kg body weight (b.w.)	Swiss albino mice Oral (by gavage)	DNA SSBs by alkaline Comet assay	Positive (1.5 up to 49.3 mg Ni/kg b.w.) (first week post-treatment)	Danadevi et al (2004)
Nickel chloride $NiCl_2$ (4.5, 9.1 and 18.1 mg Ni /kg b.w.)	Mice	CA and MN	Positive Dose-related	Dhir et al. (1991)
Nickel chloride $NiCl_2$ 43.02 mg Ni /kg) Nickel sulphate $NiSO_4$ 27.68 mg Ni /kg) Nickel nitrate $NiNO_3$ 23.20 mg Ni /kg	Mice	CA and MN	Positive 23.20 up to 43.02 mg/kg Ni b.w	Sobti and Gill (1989)
Nickel sulphate hexahydrate $NiSO_4 \cdot 6\ H_2O$ 27.9, 55.8 and 111.7 mg Ni/kg b.w. per day)	Mice (by gavage)	MN	Negative	Oller and Erexson (2007)

CA: chromosomal aberration; MN: Micronucleus; SSB: single-strand break.

7.2.4.3. Genotoxic effects in humans

DNA damage and chromosomal alterations have been analysed in cells from Ni-exposed workers with inconsistent findings. Examples of positive and negative studies are provided below.

Kiilunen et al. (1997) analysed the genotoxic effects of Ni exposure in workers of an electrolytic Ni refinery by measuring micronuclei frequency in smears from the buccal mucosa. At the time of measurement the urinary concentrations of Ni in workers were 0.1–2 micromol/L. The frequency of micronucleated epithelial cells in the buccal mucosa of Ni refinery workers was not significantly elevated by comparison with referents. No relationship was observed between micronucleus frequencies and levels of Ni in air, urine or blood.

Werfel et al. (1998) reported elevated DNA SSB and SCE frequencies in lymphocytes of welders exposed to chromium and Ni although in this case it is not possible to assign the effects solely to Ni.

A cross-sectional study including 824 participants was conducted from 1993 to 1994 in an urban population in Germany to investigate the association between metal exposure and oxidative DNA damage (Merzenich et al., 2001). Chromium, cadmium, and Ni were measured in urine samples and lead was determined in blood samples. The concentrations of metals indicated a low body load (in the case of Ni, median values: 1.0 µg Ni/L urine). A positive association between Ni levels and the rate of oxidative DNA lesions (Fpg-sensitive sites) was observed (odds ratio, 2.15; tertiles 1 *versus* 3, $P < 0.05$).

In a population study conducted by Danadevi et al. (2004) welders and an equal number of control subjects were monitored for DNA damage in blood leucocytes utilizing the comet assay and a few subjects were randomly selected for estimation of Cr and Ni content in whole blood by inductively coupled plasma mass spectrometry. Welders had higher Cr and Ni content when compared with controls (in the case of Ni 132.39 *versus* 16.91 µg/L; $P < 0.001$) and a larger mean comet tail length than that of the controls. In addition, the micronucleus test on buccal epithelial cells was carried out in a few randomly selected subjects and welders showed a significant increase in micronucleated cells compared with controls. Therefore occupational exposure had a significant effect on DNA mean tail length, but whether this is due to chromium and/or Ni exposure cannot be answered.

A study was conducted to determine both the genotoxicity of Ni in buccal epithelial cells and the urinary excretion of Ni in children ($n = 37$) with metal crowns (Morán-Martínez et al., 2013). Micronuclei assays were performed using buccal cells from 37 patients, and Ni levels were determined from urine samples using inductively coupled plasma mass spectrometry at 1 (basal value), 15, and 45 days following the placement of crowns in each patient. Ni urinary excretion levels and the frequency of exposed micronuclei increased significantly between 1 and 45 days post-crown placement.

7.2.4.4. Conclusions

There is considerable evidence for the induction of DNA damage by soluble Ni compounds both *in vitro* and *in vivo*. Various types of DNA damage have been reported including DNA SSBs, oxidative base damage and DNA protein crosslinks. The formation of hydroxyl radicals by Ni is strongly suggested as the first step in the formation of all types of Ni-induced DNA lesions and the inhibition of DNA repair (caused by Ni compounds) may account for their persistence.

As far as mutagenicity is concerned soluble Ni compounds are negative in bacterial cells and, in general, weak mutagens in mammalian cells. It should be noted that most of the evidence for Ni mutagenesis in mammalian cells was obtained using transgenic cell lines where the effects were shown to be related to the integration of the transgene into a heterochromatic region of the genome (Klein et al., 1994) or to methylation of the transgene (Lee et al., 1995). Soluble Ni compounds can induce morphological transformation of mammalian cells *in vitro*.

Chromosomal effects due to both aneugenic and clastogenic activity of soluble Ni compounds have been observed *in vitro*. Interestingly, compared to active euchromatic regions transcriptionally inactive heterochromatic regions have been shown to be more susceptible targets to chromosomal breaks (Conway and Costa, 1989). The evidence for *in vivo* induction of chromosomal alterations is inconsistent. In particular, for oral studies, old studies, which are not compliant with current guidelines, indicate positive effects for micronuclei induction whereas a more recent, well conducted (but single) study indicates lack of clastogenic effects.

In conclusion, the complexity of the genotoxic effects of Ni compounds likely reflect the multiple mechanisms that mediate Ni-induced carcinogenesis including ROS production, inhibition of DNA repair, hypoxia-mimicking effects, dysregulation of cell signalling and alterations of the epigenetic landscape (see mode of action). On the basis of the current data, the genotoxicity of the Ni compounds is likely due to indirect effects.

7.2.5. Carcinogenicity

In 1943 Campbell reported that chronic inhalation of Ni dust caused a two-fold increase of lung tumour incidence in mice. Since then in view of the evidence in humans and experimental animals for the carcinogenicity of Ni compounds and Ni metal, IARC concluded that 'Nickel compounds are carcinogenic to humans (Group 1)'. Ni compounds have been shown to induce tumours in experimental animals with particulate Ni compounds with intermediate solubility like Ni_3S_2 showing a high carcinogenic potential and soluble Ni(II) salts relatively weaker effects (IARC, 2012). The difference in carcinogenic activity has been ascribed to the different diffusion and transportation within the cells depending on the solubility of Ni compounds (see Section 7.1.1). The routes of administration that were shown to produce tumors include inhalation, intramuscular and subcutaneous administration and intraperitoneal, intrarenal, intratesticular and intraocular injection. It has been suggested that because soluble Ni compounds have the highest bioaccessibility in gastric fluids and the highest systemic absorption compared to insoluble Ni compounds, therefore the soluble Ni compounds would present the highest potential for systemic carcinogenicity after oral exposure. However, no tumours were found in animals that received soluble Ni compounds by oral administration.

Oral studies will be addressed in detail because of the relevance of this route of administration for this opinion.

7.2.5.1. Oral carcinogenicity in experimental animals

Several studies performed in the 1960–1980s have addressed the carcinogenicity of water soluble Ni compounds by oral exposure (Schroeder et al., 1964, 1974; Schroeder and Mitchener, 1975; Ambrose et al., 1976; Kurokawa et al., 1985) and, although these studies were all deficient in some aspect, they did not show evidence of carcinogenicity.

Ni sulphide hexahydrate was tested by oral gavage in a 2-year (104 weeks) study (Heim et al., 2007) in male and female Fischer 344 rats at exposure levels of 10, 30 and 50 mg/kg. A statistically significant and exposure related reduction in b.w. in both males and females was reported at 30 and 50 mg/kg/day. In high dose females (but not males) an exposure related increased mortality was observed. No exposure-related increase in tumour frequency was observed. Only one tumour type, keratoacanthoma (tail), was significantly increased in males at 10 mg $NiSO_4 \cdot 6\ H_2O$/kg b.w per day but not at higher doses as compared to untreated rats. This is a common tumour type and there was no exposure-response relationship and therefore does not support the carcinogenicity of orally-administered soluble Ni.

Ni chloride was tested for carcinogenicity in female hairless mice (CRL:SK1-hrBR). The mice were exposed to ultraviolet radiation (UVR) (1.0 kJ/m^2, three days per week) for 26 weeks either alone or in combination with 20, 100 or 500 mg/L Ni chloride in drinking water. The concentrations of Ni chloride had no effect on growth of the mice compared to control mice. Mice treated with 100 and 500 ppm Ni chloride significantly increased the skin Ni levels. In female mice UVR alone induced 1.7 +/- 0.4 cancers/mouse and the addition of 20, 100 or 500 mg/L Ni chloride increased the yields to 2.8 +/- 0.9, 5.6 +/- 0.7 and 4.2 +/- 1.0 cancers/mouse, respectively. Therefore, Ni acts as a co-carcinogen with UVR by increasing the UVR-induced skin tumour incidence.

7.2.5.2. Immunotoxicity including sensitisation

Immunologic responses to Ni constitute a two-edged sword. On the one hand, Ni is a sensitizer, and specific immune responses to Ni result in adverse hypersensitivity reactions. On the other hand, Ni can also have direct toxic effects on the immune system, resulting in dysregulation and subsequent compromised resistance.

7.2.5.3. Sensitization

There is evidence that combination of Ni with circulating or tissue protein gives rise to new antigens. These antigens can act as contact allergen and cause sensitization, that is either expressed as Type I or

Type IV hypersensitivity, mediated by reagins and allergen-specific T lymphocytes, expressing in a wide range of cutaneous eruptions following dermal or systemic exposure.

Sensitizing activity of Ni has been shown in classical tests to predict such activity of chemicals, i.e. the Guinea Pig Maximization Test (Modjtahedi et al., 2011). Oral exposure studies to investigate sensitization to Ni by the oral route, or studies in which sensitized animals are orally exposed are scant. Administration of water enriched with Ni chloride in mice prevented subsequent dermal sensitization to Ni (Artik et al., 2001) and showed that invariant natural killer T cells (iNKT cells) are required for the induction of oral tolerance towards Ni. This was in contrast to dermal Ni sensitization, in which these cells do not appear to play a role (Roelofs-Haaruis et al., 2004).

7.2.5.4. Direct toxicity to the immune system

Ni has been shown to stimulate the immune system, inducing maturation of T lymphocytes from virgin into memory cells; these latter cells seem to accumulate in the intestinal mucosa (Di Gioacchino et al., 2000). Such stimulation may be a consequence of allergic responses to Ni, but may potentially also have consequences for regulation of immune functions that are not related to Ni itself, but that are induced by other antigens such as may occur in the intestinal tract, i.e. Ni may in fact also act as an adjuvant of immune reactions to antigen that are not related to Ni, leading to enhanced responses to such non-related antigens.

The effects of Ni on the humoral immune response were studied by assessing effects on specific IgM antibody production against sheep red blood cells (SRBC) and polyclonal IgG antibody production in the spleens of mice intraperitoneally injected with Ni chloride (Nagai et al., 1989). The conclusion of that study was that the allergenicity of Ni is more pronounced than its immunomodulatory influence. Effects on antibody responses were also not observed by Smialowycz et al. (1987), who investigated potential immunotoxicity in Fischer 344 rats following a single intramuscular injection at doses ranging from 10 to 20 mg/kg b.w. Mitogen responsiveness of splenic lymphocytes were not affected either. On the other hand, natural killer (NK) cell activity was significantly suppressed in rats injected with 10, 15, or 20 mg/kg $NiCl_2$. NK cell suppression was observed in both male and female rats and for both allogeneic W/Fu-G1 target cells as well as xenogeneic YAC-1 target cells. Ni-induced suppression of NK activity was transient, with levels returning to control values within three days following treatment. The relevance of this Ni-induced suppression of NK activity was manifested by an increase in mortality of rats injected with MADB106 tumour cells (a mammary adeno carcinoma cell line, which has been used often in tumour transplantation studies to investigate host resistance).

Effects on NK activity were further corroborated by *in vitro* studies: *In vitro* exposure of $NiCl_2$ for 2 hours to spleen cells of female Sprague- Dawley rats and male/female cynomolgus monkeys resulted in statistically significant decreases in NK cell activity of both species. D'Antò et al. (2009) reported that also the viability of murine macrophages was reduced following *in vitro* exposure to $NiCl_2$.

The *in vitro* effects of Ni chloride on NK cell activity were also compared between the rat and the cynomolgus monkey (Condevaux et al., 2001). At the higher concentration, Ni chloride induced a significant decrease in NK cell activity in the ranges of 21.6–24.3 % (rat) and 34.4–42.2 % (monkey), depending on the effector-to-target cell ratio used.

Suppression of immune functions was also observed by Harkin et al. (2003) in a dietary exposure study. Dietary exposure of male and female Wistar rats to $NiCl_2$ resulted in dose- and time-dependent immunosuppression effects on T-lymphocyte proliferation and Th1 (IFN-gamma) and Th2 (IL-10) cytokine production. Production of the pro-inflammatory cytokine TNF-alpha was inhibited in a dose dependent manner. There was a dose-dependent increase in the production of the anti-inflammatory cytokine IL-10 from lipopolysaccharide (LPS) stimulated cultures. Minimal plasma concentrations of Ni (209–585 ng/mL) were required to provoke immunosuppression.

As host resistance assays against experimental infections are generally considered as the most relevant criteria when predicting the immunotoxicity of drugs and chemicals, the effects of Ni chloride on the

resistance toward experimental *Klebsiella pneumoniae* infection was investigated in mice, with particular emphasis on the interference of the time of toxic exposure with the infectious challenge. Interestingly, one single intraperitoneal dose of 4 mg/kg Ni enhanced the resistance of mice against *Klebsiella pneumoniae* when administered 24 hours before the infectious challenge, whereas host resistance proved to be impaired when the same dose was injected five hours after the infectious challenge. Ni appear to exert complex and possibly opposite effects on antibody response and phagocytosis, it remains to establish which immunotoxic consequences if any, an acute or chronic exposure to these heavy metals is likely to have in man (Laschi-Loquerie et al., 1987).

It should be noted that natural killer activity, which is the immune function that seems especially affected by Ni exposure, is not the most prominent defence mechanism for *Klebsiella*, other than resistance to MADB106 tumour cells, mentioned earlier (Smialowycz et al., 1987), in which model NK activity does play a more prominent role.

7.3. Observations in humans

The general population is primarily exposed to Ni via food and drinking water, whereas inhalation from ambient air and percutaneous absorption are generally minor sources of exposure.

A subpopulation of possibly higher exposure to Ni by other sources than by food are workers in Ni producing and related industries exposed to airborne fumes, dusts and mists containing Ni and its compounds (NTP, 2000; IARC, 2012). The carcinogenic risk of Ni through inhalation has been characterized most recently by IARC, see Section 1.3. Notably, for cancer on other sites than lung and nasal sinus no consistent epidemiological/occupational data have been identified.

Another subpopulation of individuals with possibly higher exposure to Ni than by food are smokers. An additional effect of cigarette smoking on the risk of developing cancer of the lung and nasal sinus has been discussed already by Doll et al. (1970) who suggested that susceptibility to cancer induction is determined by the amount of previous exposure to other agents and that the reduced risk of lung cancer in some subpopulations could be due to reduced heavy cigarette smokers if the effects of cigarette smoking and specific occupational hazards interact. Andersen et al (1996) investigated the relation between occupational among Ni refinery workers and their exposure to different forms of Ni over time and the interaction between smoking and total exposure to Ni. From the results of a study of more than 4 700 workers a multiplicative effect of smoking and Ni exposure was suggested, when the RR for exposed workers who had never smoked was 1.1 (95 % CI 0.2–5.1) in contrast the RR of 5.1 (95 % CI 1.3–20.5) for exposed workers who smoked. It has been estimated that cigarette smoke may contribute rather more than ambient air to daily absorption of Ni by inhalation if about 0.2 µg Ni is inhaled from each cigarette. However, it was also noted by EU RAR (2008) that other harmful chemical agents present in cigarette smoke (particulate matter, polyaromatic hydrocarbons, benzene, nicotine etc.) contribute much more to human health problems of smokers than extra Ni exposure due to cigarette.

This chapter will not detail human health effects from exposure to Ni from occupational exposure or exposure from ambient air, including cigarette smoke but will concentrate on the investigation of non-carcinogenic health effects in humans including contact dermatitis, (occupational) asthma and systemic effects (e.g. respiratory, gastrointestinal, haematological, musculoskeletal and, hepatic, renal and ocular effects), but also immunological, neurological, reproductive and developmental effects, including death after high exposure, that have been associated with human exposure to Ni through oral ingestion.

Human health effects distinguished by route of exposure have been described recently by ATSDR (2005), WHO (2007), OEHHA (2011) and IARC (2012).

7.3.1. Human health effects

Ni and Ni compounds have been classified by IARC as carcinogenic to humans (Group 1) causing cancers of the lung, nasal cavity and paranasal sinuses after inhalation. There is currently no consistency in the epidemiological data to suggest that Ni compounds cause cancer at additional sites or by additional routes.

No human data were identified on respiratory, endocrine, metabolic, ocular, neurological, and carcinogenic effects after oral exposure.

In this section the studies reporting an association between gastrointestinal effects and oral exposure to Ni are summarized. Regarding reproductive and developmental effects in humans the studies presented below refer to populations exposed to Ni by inhalation although a partial exposure by ingestion cannot be excluded.

7.3.1.1. Gastrointestinal effects

Gastrointestinal (GI) (vomiting, cramps, and diarrhea) and neurological symptoms (giddiness, headache, and weariness) were the most reported effects after acute exposure.

Symptoms of gastrointestinal distress were reported for workers who drank water during one work shift from a water fountain contaminated with Ni sulphate, Ni chloride, and boric acid (Sunderman et al., 1988). Thirty-five workers were exposed, 20 reported symptoms (estimated dose of 7.1–35.7 mg Ni/kg), and 10 of them were hospitalized. The symptoms included nausea (15 workers), abdominal cramps (14 workers), diarrhea (4 workers), and vomiting (3 workers). The investigators noted that the intake of boric acid probably did not contribute to the observed effects .The same publication reports a transient increase in blood reticulocytes in workers who were hospitalized as well as renal toxicity (increase of serum bilirubin and urinary albumin) effects. These authors reported also neurological effects that included giddiness (n = 7), weariness (n = 6), and headache (n = 5).

Human health effects, in particular gastrointestinal effects, from intoxication by high amounts Ni in few or single cases after oral exposure, or exposure via other routes (usually inhalation) where some ingestion of Ni cannot be excluded, were described in eight case reports, see Appendix G

Picarelli et al. (2011) investigated the performance of a novel oral mucosa patch test in a small cohort of 86 patients (including 18 with celiac disease and 13 with lactase deficiency) presenting intestinal and extra-intestinal symptoms possibly related to the ingestion of Ni-containing food (e.g. abdominal swelling, abdominal pain, diarrhea, constipation and stomatitis). Out of the 86 patients, 33 had positive results when tested for hypersensitivity to Ni via a standard epicutaneous patch test, and 55 were positive at the oral mucosa patch test. The authors also noted that the severity of the reported GI symptoms was higher in patients testing positive at the oral mucosa patch test and concluded that a close relationship between Ni intake and intestinal symptoms commonly reported by Ni-sensitive patients was present in the study.

7.3.1.2. Reproductive and developmental effects

A first epidemiological study on reproductive and developmental effects in humans is that of Chashschin et al. (1994) who reported 15.6 % spontaneous abortions among 290 women working in a Ni hydrometallurgy refining plant in Russia in the arctic region beyond the Polar Circle, compared to 8.5 % incidence in 336 female construction workers supposed to be without any occupational Ni exposure as controls. Exposure, primarily to Ni sulphate was estimated as of 0.11 to 0.31 mg Ni/m^3 in the air in that plant and partial exposure by ingestion could not be excluded In the same study, the authors also noted a statistically significant increase in structural malformations among offspring born to 356 workers (16.9 %) compared to 342 controls (5.8 %) and increased relative risks of 6.1 for cardiovascular, and 1.9 for musculoskeletal defects, respectively. Heavy manual activity and heat stress of the exposed women was noted as potential confounders (see also OEHHA, 2011). This study was criticized at several instances as of being inconclusive due to flaws in the study design and

reporting (EU RAR, 2008; chapter 4.1.2.8.2). In a follow-up register-based cohort study Vaktskjold et al. (2006) investigated whether pregnant women employed in Ni-exposed work areas are at elevated risk of delivering a newborn with a genital malformation. Therefore, they used data on pregnancy outcomes and occupational information from the Kola Birth Registry in Russia. Each woman giving birth in the period March 1973 through 2001 was assigned a categorical Ni exposure rating reflecting the occupation at the time of becoming pregnant, using personal monitoring data of the water-soluble Ni subfraction of the inhalable aerosol fraction or the measured urinary Ni concentrations. The reference population comprised delivering women from Moncegorsk with a background exposure level. The study cohort comprised 23 141 live- or stillborn infants from a total of 24 534 deliveries. Exposure was classified into the three categories of background, low and high exposure (< 10, 10 to < 0, and ≥ 70 µg/L). The odds ratio for Ni-exposed women delivering a newborn with a genital malformation was 0.81 [95 % confidence interval (95 % CI): 0.52–1.26], and that for an undescended testicle was 0.76 (95 % CI: 0.40–1.47). The authors concluded that no adverse effect of maternal exposure to water-soluble Ni was found but noted that there were only few cases in the higher exposure groups. In a second study on 22 836 births (> 27 weeks of gestation) on possibly elevated risk of delivering a newborn small-for-gestational-age (SGA defined as below the 10th percentile birth weight for gestational age in the source population) Vaktskjold et al. (2007) found also no adverse effect of maternal occupational exposure to water-soluble Ni in the first part of pregnancy for newborns without trisomy. The adjusted odds ratio for Ni-exposed women for giving birth to an SGA newborn was 0.84 (95 % CI: 0.75–0.93). Furthermore, Vaktskjold et al. (2008a) investigated the risk of spontaneous abortion in the same geographical area in a case-control study. The unadjusted odds ratio for the association between the maternal exposure to Ni and a spontaneous abortion for Ni-exposed women was 1.38 (95 % confidence interval: 1.04–1.84); when adjusted for maternal factors it was 1.14 (0.95–1.37) and as such not statistically significant. The authors concluded that there was no association between maternal occupational exposure to water-soluble Ni in early pregnancy and the risk of self-reported spontaneous abortion, although the findings would not exclude the possibility of a weak excess risk, or a risk in the first weeks of pregnancy. Maternal smoking had some but not statistically significant effect (OR 1.15, 95 % CI: 0.96–1.39). Another study of this group (Vaktskjold et al., 2008b) analysed the incidence of musculoskeletal defects in the offspring in the cohort described above and observed among 22 965 births, 304 infants (13.3/1 000 births; 95 % C.I. 11.9–14.7) diagnosed with isolated musculoskeletal defects(s) concluding that despite the high incidence of defects there was no apparent association (adjusted OR 0.96, 95 % C.I: 0.76–1.21) with maternal Ni exposure. The Panel concluded that available data from these case-control studies do not support the existence of an association between oral exposure to Ni and reproductive and developmental effects in humans.

Danadevi et al. (2003) examined semen quality of 57 workers from a welding plant in South India and 57 controls in relation to blood Ni and chromium concentrations using ICP-MS. Twenty-eight male welders and 27 control men were selected randomly from the total number of subjects for blood sampling and the blood Ni level of the exposed workers was 123.3 ± 35.2 µg/L, significantly higher than that of the controls (16.7 ± 5.8 µg/L). Sperm concentrations of exposed workers were 14.5 ± 24.0 millions/mL and those of the control group were 62.8 ± 43.7 millions/mL. Rapid linear sperm motility was decreased in exposed workers compared to controls and there was a significant positive correlation between the percentage of sperm tail defects and blood Ni concentration in exposed workers. More abnormal characteristics were found in the semen of exposed workers. Semen abnormalities correlated with the number of years of exposure to welding fumes containing Ni and chromium. The Panel noted that the study was limited by the size and a possible selection bias of the cohorts and the fact that exposure to Ni was determined only for a subset of workers using a single measure of the concentration of Ni in blood in presence of other heavy metals.

Figá-Talamanca and Petrelli (2000) studied the gender ratio among children of men differently exposed to metal fumes of Ni and Cr (n = 48 in administration, n = 74 technicians, n = 31 stampers and n = 63 founders) in an Italian mint and observed a statistically significantly reduced portion of male children in founders compare to administrative working persons and the general population. This finding is in contrast to the results from a large Danish cohort of more than 10 000 metalworkers

where no change in the gender ratio was found for children at risk from paternal welding where workers can be exposed to high levels of chromium and Ni (Bonde et al., 1992).

In summary, and allowing for the uncertainty on the level of exposure to Ni by ingestion, the CONTAM Panel noted that the results of these studies do not support the association of effects on reproduction and developmental with oral exposure to Ni.

7.3.2. Sensitization

Allergic contact dermatitis, i.e. type IV hypersensitivity, is the most prevalent effect of Ni in the general population (Hostynek, 2006). In the USA, Ni allergic contact dermatitis has an incidence of 14.3 %, and is on the rise from 10 years ago, when the incidence was 10 %. Similar figures were reported by Schnuch et al. (2002), who reviewed information from EU, Asia and USA, and by Mortz et al (2013), reporting on a cohort study of 1 501 8th grade school children, that lasted 15 years, and in which Ni sensitization was observed in 11.8 % of the study group.

A rise in Ni sensitization has been presumed to represent an increased exposure to Ni in the environment-especially in costume jewellery and belt buckles (Silverberg et al., 2002). Occupational exposure to Ni can cause allergic asthma via type I allergic reactions in which serum from affected individuals shows specific IgE antibodies against serum albumin conjugates (Kusaka, 1993).Very few cases of immediate contact urticaria to Ni have been reported. Whereas Type I immune responses may be underlying such conditions, it has also been postulated that Ni may act as a mast cell discharger on a non-immunological basis (Walsh et al., 2010).

Consumption of Ni-rich diet may elicit eczematous flare-up reaction in the skin in sensitized individuals, a phenomenon called systemic Ni contact dermatitis (SCD) or haematogenous contact eczema (Erdmann and Werfel, 2006; Jensen et al., 2006). Indeed, ingested Ni may have consequences for the expression of skin conditions in sensitized individuals, such as flare-up of cutaneous reactions in some Ni-allergic patients (Christensen and Möller, 1975; Kaaber et al, 1978; Cronin et al., 1980; Veien et al., 1983; Hindsén et al., 2001; Gangemi et al., 2009). It should also be noted that on the other hand, experimental studies have also shown that repeated oral exposure to Ni may prevent diminish sensitization. Sjövall et al. (1987), Santucci et al. (1988), and Bonamonte et al. (2011) reported reduction of Ni contact dermatitis after oral exposure to soluble Ni over a longer period of time.

In a study by Nielsen et al. (1999) a stable Ni isotope, ^{61}Ni, was given in drinking water to 20 Ni sensitized women and 20 age-matched controls. The subjects were fasted and had an empty stomach. Both groups had vesicular hand eczema of the pompholyx type. Nine of 20 Ni allergic eczema patients experienced aggravation of hand eczema after oral Ni administration, and three also developed a maculopapular exanthema. No exacerbation was seen in the control group. A LOAEL of 12 µg/kg of b.w. was established after provocation. The guideline value for Ni in drinking water established by WHO (2005) is based on this study.

Jensen et al. (2006) performed a meta-analysis study on Ni exposure investigations to provide the best possible estimation of threshold values of Ni doses that may cause systemic contact dermatitis in Ni-sensitive patients. The authors identified 17 investigations to study the dose relationship of responses to oral exposure to Ni in Ni-sensitive individuals (Christensen and Möller., 1975; Kaaber et al., 1978, 1979; Jordan and King; 1979; Veien and Kaaber, 1979; Cronin et al, 1980; Burrows et al., 1981; Bedello et al., 1985; Sertoli et al., 1985; Gawkrodger et al., 1986; Roduner et al., 1987; Veien et al., 1987; Santucci et al., 1988; Möller et al., 1999; Nielsen et al., 1999; Hindsén et al., 2001; Jensen et al., 2003). There appeared a clear indication of increasing reaction rate associated with increasing dose. Of these 17 studies, some were excluded for several reasons. Some studies had no placebo controls (including the study by Nielsen et al. (1999) on which WHO based its guideline value for Ni in drinking water. Other studies were excluded because of positive responses in placebo groups. Some studies investigated double exposures. Only studies that investigated single exposures were selected for dose response analysis. Statistical analyses were performed in a stepwise procedure of nine studies

that were eventually selected, and that comprised a total of 171 patients that were included in the final dose–response analysis. The studies were divided into a homogenous middle group of five studies and two groups of two studies with a higher and lower response frequency, respectively, described by logistic dose–response curves shifted in parallel. On the basis of these curves, calculations were made of the doses that, theoretically, would cause systemic contact dermatitis in exposed Ni-sensitive patients. On the basis of this meta-analysis, theoretical exposure doses predict that oral Ni exposure with 0.22 mg, 0.35 mg, or 0.53 mg (depending on the dose response curve used) will make 1 % of Ni-sensitive individuals respond. Similarly, 10 % of these patients will react if they are exposed orally to Ni doses of 0.55 mg, 0.87 mg, or 1.33 mg. The results from the two most sensitive groups show that 1 % of these individuals may react with systemic contact dermatitis at normal daily Ni exposure from drinking water and diet, i.e. 0.22–0.35 mg Ni. The CONTAM Panel noted difficulties with accepting this meta-analysis as a basis for deriving a health-based guidance value for acute exposure to Ni. The authors had excluded some studies which exhibited a clear internal dose-response relationship and had included studies for which no internal dose-response relationship could be assessed (e.g. when only one exposure level has been used in the challenge). A reason for excluding studies showing a positive dose response was that some positive effects at dose equal to zero (placebo responses) were noted. However, the Panel considered that it would be appropriate to include these studies, as including studies with background response is the rule in dose-response analyses. In addition, the division in this meta-analysis of the study population in very sensitive, medium sensitive, and less sensitive groups was arbitrary and not underpinned by scientific argumentation.

The CONTAM Panel examined all single 17 studies mentioned in the review by Jensen et al. (2006) for suitability for dose-response analysis individually and identified three such studies. Gawkrodger et al. (1986) investigated 24 persons (22 females and 2 males) positive in patch testing to Ni sulphate, administering Ni salt in lactose. Hindsén et al. (2001) challenged 30 females (12 with atopy and pompholx and 18 without atopy and hand eczema) fasting after midnight to Ni sulphate in lactulose. Jensen et al. (2003) investigated 40 Ni-sensitive individuals (39 female, 1 male) that were positive in patch testing to Ni. The patients were exposed to Ni sulphate hexahydrate in lactose capsules as single bolus in the morning after a 12 hours fasting period. No other dietary intervention was conducted, hence each individual was exposed to Ni in the three dose groups or placebo (lactose) in the control group in addition to the Ni exposure from the normal diet in this study. Exposure from diet was not estimated and one day after the oral exposure the status of the skin area previously exposed to patch testing with Ni was scored for objective clinical responses.

Of these studies, the study by Jensen et al.(2003) showed effects at the lowest doses, with incidences of 1/10, 4/10, 4/10 and 7/10 at the doses 0, 0.3, 1, and 4 µg Ni per person,

7.3.2.1. Direct toxicity to the immune system

Findings in animals concerning effects of Ni on host resistance were corroborated by studies in humans. Studies by Salsano et al. (2004) and by Verna et al. (2005) showed a clear difference in the NK cell activity between Ni-tolerant and intolerant individuals. In another study the incidence of different infectious diseases in 100 patients with Ni hypersensitivity in comparison to 100 matched volunteers with negative European standard patch test as healthy controls was investigated (Rosato et al., 2009). In patients with Ni hypersensitivity a higher incidence of recurrent herpes labialis, urinary tract infections, genital candidiasis, and upper respiratory tract infections was detected. Fifteen patients with Ni allergic hypersensitivity followed a Ni-poor diet. After a one-year diet a net reduction of incidence of recurrent herpes labialis was found. The number of episodes of recurrent herpes labialis (RHL) per year decreased from 6 +/- 2.75 to 2.4 +/- 1.2.

7.3.2.2. Systemic Nickel Allergy Syndrome

Whereas contact allergy is the most frequent clinical pattern in Ni-sensitized individuals, and resistance to infections may be influenced, many other clinical elements may demonstrate that the systemic absorption of Ni, e.g. by the oral route, is able to elicit gastrointestinal (e.g. abdominal pain, diarrhoea and/or constipation, nausea and/or vomiting), atypical systemic manifestations (e.g.

headache, chronic fatigue) and chronic dermatological symptoms (e.g. urticaria-angioedema), that are called Systemic Nickel Allergy Syndrome (SNAS). Whereas the relationship between acute contact dermatitis (ACD) and contact with Ni is undisputed and widely confirmed in literature, the situation is different for SNAS. The occurrence of SCD as a systemic reaction to the Ni normally assumed in the daily diet is very controversial. In particular, further and larger studies are needed to assess the reality and the prevalence of Ni urticaria. With respect to Ni-related gastrointestinal symptoms, as well as chronic fatigue syndrome, fibromyalgia, headache, recurring cold sores and recurrent infections in general, the data available in the literature are not conclusive and the studies lack the support of clear, first-hand evidence. With respect to respiratory disorders, the role of food Ni and the effectiveness of a dietary treatment have been assumed but not proven. In fact, the usefulness of a therapeutic low-Ni diet is controversial: rare, if not exceptional, and limited to very sporadic cases of SCD. Additionally, the quantitative and qualitative composition of a low-Ni diet presents few certainties and many uncertainties. The low-Ni diets suggested in literature are highly variable, both in the extension of the restrictions and in their details-and the differences are not marginal. The current information that is available about SNAS and its relationship with oral Ni exposure does not allow to draw final conclusions and further and broader studies, more rigorously conducted, are needed.

7.3.3. Conclusions

The general population is primarily exposed to Ni via food and drinking water, whereas inhalation from ambient air and percutaneous absorption are generally minor sources of exposure. Subpopulations of possibly higher exposure are workers in Ni producing and related industries when exposed to airborne fumes, dusts and mists and smokers. Although a multiplicative effect of smoking and Ni exposure has been suggested other harmful chemical agents present in cigarette smoke contribute much more to human health problems of smokers than the extra Ni exposure.

Ni and Ni compounds have been classified by IARC as human carcinogens causing cancers of the lung, nasal cavity and paranasal sinuses after inhalation. Based on i) the lack of epidemiological data suggesting that Ni compounds cause cancer at additional sites or by additional routes, ii) the lack of tumours in the oral carcinogenicity studies in experimental animals and, iii) the modes of action, the CONTAM Panel considered it unlikely that dietary exposure to Ni results in cancer in humans.

Non-carcinogenic health effects of oral exposure to Ni include contact dermatitis and systemic effects on the gastrointestinal, haematological, neurological and immune system. Gastrointestinal (vomiting, cramps, and diarrhea) and neurological symptoms (giddiness, headache, and weariness) were the most reported effects after acute exposure.

Exposure through skin or airways may lead to Ni sensitization. Combination of Ni with circulating or tissue protein gives rise to new antigens and act as contact allergen and cause sensitization. Alternatively, binding to MHC and or MHC-bound peptides and T cell receptors leading to the activation of NI-specific T cells may result in sensitization. Whereas oral exposure to Ni may not readily lead to sensitization, oral absorption of Ni is able to elicit eczematous flare-up reactions in the skin in Ni-sensitized individuals.

Patients with severe Ni sensitization constitute a particular sensitive population to oral challenge with Ni and are potentially at risk from excessive exposure to Ni in food and water the data available could be considered to derive an RP for systemic contact dermatitis elicited in Ni-sensitive humans after acute oral exposure to Ni as the worst case scenario.

7.4. Biomonitoring

Plasma and urine concentrations of Ni are influenced by the chemical and physical properties of the Ni compound studied, and by the time of sampling (usually at the end of a working shift), and the analytical methods used. Elevated levels of Ni in biological fluids and tissue samples only indicate uptake of Ni, and may not correlate directly to exposure levels. Nor can they be used to identify the absorption route.

In occupational settings, plasma and urine concentrations of Ni are useful biomarkers of Ni inhalation exposure on a group basis (Sunderman et al., 1986). The levels in plasma and urine are highly dependent on the Ni species in air. Less soluble compounds, such as oxidic and sulphidic Ni, give relatively lower plasma and urine values than a corresponding level of soluble chlorides or sulphates, but higher values in the nasal mucosa and probably also in the lungs (possible target organs). Moreover, the correlation between exposure and biological values on an individual basis is low and significant only in some investigations involving exposure to soluble compounds. Based on an extensive review of biological monitoring data, Sunderman (1993) concluded that serum and urine Ni levels were the most useful biomarkers of Ni exposure. However, with the exception of Ni carbonyl, a relationship between Ni levels in body fluids and a specific health risk could not be established.

Levels of Ni in urine and serum can provide information about levels of Ni exposure if the route, sources, and duration of exposure are known, if the chemical identities and physical-chemical properties of the Ni compounds are known, and if physiological information (e.g. renal function) of the exposed population is available (Sunderman, 1993). Urine and serum levels of Ni in workers inhaling soluble Ni compounds reflect the amount of Ni absorbed in the previous 1 or 2 days (Sunderman et al., 1986). With respect to monitoring Ni following exposure to soluble compounds, the best correlations between exposure concentration and urine levels were found with 'end-of-shift' urine sampling (Bernacki et al., 1980) or 'next morning' urine sampling (Tola et al., 1979).

In the general population, average Ni concentrations in serum and urine are 0.2 and 1 –3 µg/L, respectively (Templeton et al., 1994). After reviewing monitoring data in occupationally exposed workers, Ohashi et al. (2006) determined reference values for Ni in urine among women of the general population of 11 prefectures in Japan. The observed geometric mean for urinary Ni was 2.1 µg/L (range, < 0.2–57 µg/L) corresponding to 1.8 µg/L (maximum, 144 µg/L) after normalization by creatinine exscretion.

The German Environmental Survey on children (GerES IV) 2003–2006 provided representative data to describe the internal Ni exposure of children aged 3–14 years in Germany. Urinary Ni levels (n = 1 576) ranged from < 0.5 to 15 mg/L, the geometric mean being 1.26 mg/L. Multivariate regression analysis showed that gender, age, socio-economic status, being overweight, consumption of hazelnut spread, nuts, cereals, chocolate and urinary creatinine were significant predictors for urinary Ni excretion of children, accounting for about 20.2 % of the variance. The main contribution (13.8 % of the variance) was accounted for by urinary creatinine concentration. No influence of Ni intake via drinking water and passive smoking was observed (Wilhelm et al., 2013).

Torjussen et al. (2003) investigated if the Ni content in inhaled smoke from commercial cigarettes and cigarettes handmade by (years-long) Ni process workers might be an additional source of Ni exposure. The measured Ni concentrations in blood plasma and urine were characterized by relatively high values and large variability, and were quite similar among smokers and non-smokers (respectively 6.2 and 48.1 µg/L in smokers and 6.4 and 50.5 µg/L in non-smokers). As expected, most tobacco Ni was recovered in the ash. It was concluded that the Ni present in the working atmosphere was probably the main source of the Ni inhaled in the workers tested. A biomonitoring study of the general population in Serbia and Montenegro showed that smokers (of commercial cigarettes) can be more exposed to Ni than non-smokers, as indicated by the significantly higher Ni urinary levels measured in smokers (< 0.01–8.20 µg/L; median, 1.20 µg/L) relative to non-smokers (< 0.01–4.60 µg/L; median, 0.50 µg/L) (Stojanović et al., 2004). A significant association was identified between smoking status – previous smokers, current light smokers, and current heavy smokers – and Ni sensitization, in that sensitization was on average higher among smokers compared with non-smokers (Thyssen et al., 2010). Another study indicated that exposure to Ni, either through the diet or by inhalation of cigarette smoke, may trigger systemic Ni allergy and contribute to syndromes of chronic fatigue and muscle pain (Regland et al., 2001).

From biological monitoring in small groups of electroplaters exposed to Ni sulphate and Ni chloride, the half-life for urinary elimination of Ni has been estimated to range from 17 to 39 hours (EU RAR,

2008). Oliveira et al. (2000) measured urinary Ni (U-Ni) in ten workers (97 samples) from a galvanizing plant that uses Ni sulphate, and in ten control subjects (55 samples) to examine the association between occupational exposure to airborne Ni and Ni absorption. Significant differences in U-Ni creatinine were seen between the exposed and control groups and between pre- and post-shift samples A significant correlation between U-Ni and airborne Ni ($r = 0.96$; $P < 0.001$) was found.

Demir et al. (2005) determined Ni concentration of the blood in 258 humans residing in a rural area exposed to cement factory emissions near Çukurhisar, a town in Eski¸sehir-Turkey and $n = 258$ controls. The physical examination of subjects did not reveal results different from those of the control group except for the diagnosis of contact dermatitis. The analyses of venous blood samples showed that Ni concentrations were in the range of the reference values of 1.0–28.0 μg/L of Painter et al. (1999) for both groups although they were higher in the exposed subjects (between 3.2 and 18.0 μg/L) compared to the controls (between 2.1 and 17.7 μg/L, $P < 0.001$).

7.4.1. Conclusion

In subjects exposed to the same species of Ni from the same absorption route, serum Ni (S-Ni) and especially U-Ni are useful biomarkers of exposure and can be used for bio-monitoring purposes, as occurs in the case of occupational setting. However, too many variables give rise to individual concentrations in biological media, which makes translation into exposure data impossible. Such variables include the bio-accessibility and bioavailability of ingested Ni, the route of entry and clearance (from the airways, the GI tract, and the skin). Once absorbed, Ni excretion rate (kinetics) depends on protein binding and renal function, which can modify both serum and urinary concentration in subjects with similar exposure. Finally, the sampling time selected to obtain blood or urinary spot samples is another variable crucial for data interpretation. As a result, it is not possible to back-calculate the contribution of intake from food or drinking water to the concentration of Ni in accessible biological media.

7.5. Modes of action

Ni can cross-link aminoacids to DNA, lead to formation of reactive oxygen species (ROS), moreover mimic hypoxia. These changes may lead to the activation of some signalling pathways, subsequent transcription factors and eventually to alterations in gene expression and cellular metabolism (Forgács et al., 2012).

7.5.1. Reproductive toxicity

Ni has been demonstrated to disturb the mammalian reproductive functions at several levels of regulation. The hormonal effects may play an important role in the reproductive toxicity of Ni both at the neuroendocrine and gonadal levels in the hypothalamic-pituitary-gonadal (HPG) axis. At the molecular level, Ni may substitute certain other metals in metal dependent enzymes, leading to an altered protein function. It readily crosses the cell membrane via calcium channels and competes with calcium for specific receptors.

In the neuroendocrine system, Ni compounds induce alterations in prolactin, LH and FSH levels (LaBella et al., 1973, Sunderman et al., 1978). Ni complexes with GnRH, and Ni-GnRH is able to bind to the GnRH receptors. The Ni-GnRH complex increases LH release in the pituitary cells. The intracellular signalling of Ni-GnRH is different from that of the native GnRH.

Ni exposure dose-dependently disturbed the regular ovarian cycle or inhibited ovulation and decreased the progesterone response to gonadotrops in rat ovary (Forgács et al., 1998).

Ni treatment decreased the implantation frequency in early embryogenesis, increased the frequency of both early and late resorptions and the frequency of stillborn and abnormal fetuses. Ni^{2+} exerts effects directly on the developing embryo/fetus (crossing the placenta), as well as indirectly by altering the maternal hormonal balance (Sunderman et al., 1978; Lu at al., 1979; Leonard and Jacquet, 1984; Mas et al., 1985; Saillenfait et al., 1993; Apostoli and Catalani, 2011).

Ni dose-dependently decrease absolute and relative testes, epididymides, seminal vesicle and prostate gland weights and reduced sperm motility and sperm count, and increased the occurrence of abnormal pathological spermatozoa (Pandey et al., 1999; Panday and Srivastava, 2000). Ni induced histopathological changes in both male (Käkelä et al., 1999; Pandey et al., 1999) and female reproductive organs (and enhanced ovarian and testicular lipid peroxidation (Doreswami et al., 2004). Ni treatment increased the frequency of localized apoptosis in the testicular interstitium, decreased the number of basal spermatogonia; reduced testicular DNA, RNA, protein content, reduced the activities of the two testicular steroidegenic enzymes 3- and 17-β-hydroxysteroid dehydrogenase (3-β-HSD, 17-β-HSD), and levels of plasma testosterone (Das and Dasgupta, 2002).

In primary gonadal cell cultures (mouse Leydig, human ovarian granulosa) Ni exposure decreased the amounts of cadherins and β-catenin along the surface of the cell-to-cell contacts, induced alterations in cell shape and distribution of microtubuli (Forgács et al., 2004; Révész et al., 2004a, b). Ni treatment produced a concentration-dependent depression in both hGC and db-cAMP stimulated progesterone production of human granulosa cells, while the cell viability remained unaltered. Similar results were found examining the testosterone production of mouse or rat Leydig cells in similar conditions (Forgács et al., 1998, 2001; Laskey and Phelps, 1991).

Ni decreased both progesterone and testosterone production of H295R cell line far below its cytotoxic concentration (Forgács et al., 2012).

As a metalloestrogen, Ni activated estrogen receptor-α (ER*α*), and the estrogenic potency of Ni was equal to estradiol in MCF-7 cell line (Martin et al., 2003).

7.5.2. Mechanisms of genotoxicity

Soluble Ni compounds are not carcinogenic when admistered to experimental animals via oral route (see section on carcinogenicity). Their genotoxic Ni activity as revealed by several the *in vitro* and *in vivo* tests is likely to be caused by indirect mechanisms. On the basis of the current literature three predominant mechanisms emerge: 1) interference with cellular redox regulation and induction of oxidative stress; 2) inhibition of DNA repair systems; 3) dysregulation of signaling pathways and alteration of the epigenetic landscape.

7.5.2.1. Oxidative stress

Treatment with soluble and insoluble Ni causes increases in reactive oxygen species (ROS) in many cell types and in animal models. ROS induction seems to be responsible of increased DNA SSBs, DNA-protein cross-links and SCEs.

Kawanishi et al. (2002) investigated the participation of ROS in Ni-induced DNA damage by examining DNA damage and site specificity of DNA cleavage induced by Ni compounds in the presence of hydrogen peroxide (H_2O_2). Incubation of calf thymus DNA with Ni(II) plus H_2O_2 induced increased levels of 8-OH-dG with increasing H_2O_2 concentration. In contrast, H_2O_2 or Ni(II) alone induced little or no 8-OH-dG increase. On the basis of these results the authors suggest that Ni(II) reacts with H_2O_2 and produces ROS causing oxidative DNA damage. In the presence of hydroxyl radical scavengers, the DNA damage decreased considerably. To estimate the site specificity of the DNA damage, ^{32}P–5′-end–labelled DNA fragments were used and treated with Ni(II) plus H_2O_2. Piperidine-labile sites were frequently induced at cytosine, thymine, and guanine residues and rarely at adenine residue. ESR studies using spin traps revealed that hydroxyl radical adducts are produced by the decomposition of H_2O_2 in the presence of Ni(II) oligopeptides (Ni(II) GlyGlyHis). These results support the speculation that reactive Ni–oxygen complexes participate in the DNA damage.

Cavallo et al. (2003) measured the ROS levels by flowcytometric analysis and DNA damage by the comet assay in human leukemic cell line (Jurkat) treated with H_2O_2 (100 µM) for 15 minutes and then allowed to recover for 4 and 24 hours, in presence or absence of $NiSO_4$ (0.017 or 0.17 µM). Cells exposed to $NiSO_4$ (0.17 µM) during the recovery time showed an inhibition of H_2O_2-induced DNA

damage repair and an increased level of ROS as compared to that induced by H_2O_2 alone. The authors hypothesize that $NiSO_4$ and H_2O_2 play a synergistic role in the reduction of the cellular antioxidant defence activities.

Chen et al. (2003a) showed a dose-dependent association between generation of ·OH radical and DNA strand breakage as determined by the comet assay in lymphocytes from healthy individuals treated with $NiCl_2$. Conversely, the induction of lipid peroxidation by $NiCl_2$ was not associated with DNA strand breaks. The authors concluded that the generation of OH radical is likely to be responsible for $NiCl_2$-induced DNA strand breakage.

In another study Chen et al. (2003b) showed that the generation of ·OH radical intermediates plays an important role in Ni-induced toxicity in human lymphocytes. Catalase, GSH and mannitol were shown to reduce the levels of Ni-induced oxidants suggesting that they may protect cells against the oxidative stress induced by Ni.

Chen et al. (2010) analysed the effects on cell cycle and apoptosis of Ni chloride ($NiCl_2$) in rat kidney cells (NRK). Data showed simultaneous concentration dependent accumulation of G2/M phase and sub-G1 phase in Ni-treated NRK cells indicating that cell cycle progression was prevented and apoptosis was induced. Induction of apoptotis was accompanied by rising levels of ROS. In conclusion, data suggested that Ni induced cytotoxicity in NRK cells involves generation of ROS, oxidative stress, DNA strand breaks, and apoptosis.

Salnikow et al. (2002) using human and rodent cells in vitro showed that acute exposure to nickel activates hypoxia-inducible transcription factor-1 (HIF-1) that is involved in the cellular responses to oxidative stress.

Oral Ni sulphate administration (2.0 mg/100 g b.w., i.p.) to Wistar male albino rats significantly increased the level of testicular lipid peroxide and decreased antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) activities and GSH concentration (Gupta et al., 2007)

Many lines of evidence have suggested that oxidative stress and inflammation play a pivotal role in the toxicity of Ni salts. Freitas et al. (2010) show that Ni(II), at sublethal concentrations, activates NADPH oxidase in human neutrophils mainly through activation of protein kinase C (PKC), thus leading to oxidative burst. In addition, Ni was shown to activate NF-κB in an NADPH oxidase dependent manner and to induce the production of IL-8 in these cells.

7.5.2.2. Inhibition of DNA repair

The treatment of cells with soluble Ni(II) increases the DNA damage and mutagenicity of several agents likely via inhibition of DNA repair (nucleotide excision repair, base excision repair and O^6-methylguanine-DNA methyltransferase).

In 1981 Loeb and Mildvan showed that the fidelity of DNA polymerase decreased in the presence of Ni(II). By interaction with proteins involved in DNA repair Ni ions could lead to co-mutagenicity (see Section 7.2.4.1.)

Hartwig et al. (1994) showed that Ni(II) interferes with the incision step in nucleotide excision repair in mammalian cells. Ni(II) was able to block the removal of cyclobutane pyrimidine dimers as determined by T4 endonuclease V-sensitive sites in UV-irradiated HeLa cells. When the alkaline unwinding technique was applied, significantly less transient DNA strand breaks after UV irradiation were detected in the presence of Ni(II) compared to UV alone, suggesting an inhibition of the incision step of nucleotide excision repair. The ligation of repair patches was also delayed in Ni-treated cells, as observed by the alkaline unwinding and nucleoid sedimentation techniques. This inhibition of DNA repair was partly reversible by the addition of magnesium(II), suggesting that the competition between Ni^{2+} and Mg^{2+} may disturb DNA-protein interactions involved in the repair process. It is of note that the repair inhibition was observed at noncytotoxic concentrations of Ni(II).

The effect of Ni(II) on the damage recognition step of the repair process was also specifically investigated by applying a gel-mobility-shift assay in HeLa nuclear extracts (Hartmann and Hartwig, 1998). Two proteins of 34 and 40 kDa were identified that bind with high affinity to a UV-irradiated synthetic oligonucleotide. When applying nuclear extracts from HeLa cells treated with Ni(II), there was a dose-dependent decrease in protein binding; this effect was largely reversible by the addition of magnesium(II) to the binding reaction. The authors conclude that Ni disturbs DNA-protein interactions essential for the initiation of nucleotide excision repair most likely by the displacement of essential metal ions.

Since some toxic metals have high affinities for -SH groups, Asmuss et al. (2000) used the bacterial formamidopyrimidine-DNA glycosylase (Fpg protein) and the mammalian XPA protein as models to investigate whether zinc finger structures in DNA repair enzymes are particularly sensitive to carinogenic metals. Ni(II) did not affect the activity of the Fpg protein significantly but reduced the DNA-binding ability of XPA. XPA is a member of the protein complex of the nucleotide excision repair pathway of DNA repair, participating in the assembly of the incision complex. Simultaneous treatment with Zn(II) prevented largely the inhibition induced by Ni(II). The authors propose that zinc finger structures may be sensitive targets for toxic metal compounds, but each zinc finger protein has unique sensitivities.

The possible molecular mechanisms of XPA inhibition were later addressed by Bal et al (2003). The 4S zinc finger domain of XPA is involved the interactions with other NER proteins. The Ni(II) interactions with the synthetic 37 peptide (XPAzf), representing the XPA zinc finger sequence were specifically investigated. The binding constants were determined using fluorescence and UV-vis spectroscopies, structural insights were provided by CD, and oxidative damage to XPAzf was studied with HPLC. The Ni(II) ion was shown to form a square planar complex with the sulfurs of XPAzf, opposed to the tetrahedral structure of the native Zn(II) complex, thus the overall zinc finger structure is lost in the Ni(II)-substituted peptide. Zn(II)-saturated XPAzf is remarkably resistant to air oxidation and is only slowly oxidized by H_2O_2 in a concentration-dependent fashion. However, the presence of just 10-fold molar excess of Ni(II) is sufficient to accelerate this process for all three H_2O_2 concentrations tested.

7.5.3. Epigenetic mechanisms

Both water-soluble and water insoluble Ni compounds are able to cause gene silencing.

7.5.3.1. DNA methylation

One of the first experiments to demonstrate Ni's influence on DNA methylation was done in the Chinese hamster cell line (G12) by showing NiS-induced silencing of the *gpt* gene and its reactivation after treatment with the demethylating agent 5-azaC. The increased DNA methylation along with the location of the gene relative to heterochromatin was associated with the silencing of the gpt gene (Lee et al., 1995; Klein and Costa, 1997).

Later, it was shown that Ni-induced heterochromatization was caused by Ni displacing magnesium in heterochromatic complexes (Ellen et al., 2009). DNA methyltransferases (DNMTs) could be signalled somehow by condensation events. Therefore, Ni causes gene silencing first by heterochromatin spreading and subsequent methylation of those genes taken into heterochromatin. If the genes silenced are tumour suppressor or senescence genes, then carcinogenesis could be a result.

Ni has been associated with the hypermethylation of a number of genes *in vivo* including the tumor suppressor genes *p16* and *p53* (Govindarajan et al., 2002). *RARβ2* and *RASSF1A* are genes that encode tumour suppressors that mediate cell growth and induce cell cycle arrest, respectively. Wistar rats given an intramuscular injection of 10 mg Ni subsulphide developed muscle tumours that showed 5′ hypermethylation of the tumour suppressor genes *RARβ2*, *RASSF1A*, and *P16* (Zhang et al., 2011).

Genome-wide DNA hypomethylation has also been reported in Ni-induced carcinogenesis. A line of human bronchial epithelial cells, 16HBE, treated with NiS for 24 hours, showed reduced fluorescence intensity in an anti 5-methylcytosine (5-mC) immunofluorescence assay. An Sss1 methylase assay confirmed that NiS-treated cells contained a lower amount of 5mC than control cells (Yang et al., 2010).

7.5.3.2. Histone modification

Modification of histones by Ni has been reported in several studies in human cells in culture and in one study in occupationally exposed subjects.

Kang et al. (2003) reported that a high concentration of $NiCl_2$ (no less than 600 μM) caused a significant decrease of histone acetylation in human hepatoma cells. This inhibition was shown to result mainly from the effect of Ni^{2+} on the overall histone acetyltransferase (HAT) activity. Moreover, the exposure of hepatoma cells to Ni^{2+} generated ROS. Co-administration of hydrogen peroxide with Ni^{2+} generated more ROS and more histone acetylation inhibition. Addition of antioxidants together with Ni^{2+}, completely suppressed ROS generation and significantly diminished the induced histone hypoacetylation.

Ke et al. (2006) showed that there are three major changes in histone modification of cells when exposed to soluble Ni compounds: (i) loss of acetylation of H2A, H2B, H3 and H4; (ii) increases of H3K9 dimethylation; and (iii) substantial increases of the ubiquitination of H2A and H2B. These effects were observed at Ni exposure conditions that had minimum effects on cell cytotoxicity. Moreover, this study demonstrated that Ni-induced transgene silencing was associated with similar changes of histone modifications in their nucleosomes. This study is the first to show that Ni compounds increase histone ubiquitination in cells.

Karaczyn et al. (2006) investigated the effect of Ni(II) on ubiquitination, of histones H2B and H2A in nuclei of cultured 1HAEo- and HPL1D human lung cells. Ni(II) stimulated mono-ubiquitination of both histones, but at high concentrations a suppression was found. The decrease in mono-ubiquitination coincided with the appearance of truncated H2B that lacks the K120 ubiquitination site. These data show that dysregulation of H2B ubiquitination is a part of Ni(II) adverse effects.

Both water-soluble and insoluble Ni compounds were shown to induce histone ubiquitination (uH2A and uH2B) in a variety of cell lines (Ke et al., 2008). Results from the *in vitro* assays demonstrated that the presence of Ni did not affect the levels of ubiquitinated histones in the ubiquitinating assay but significantly prevented loss of uH2A and uH2B in the deubiquitinating assay, suggesting that Ni-induced histone ubiquitination is the result of inhibition of (a) putative deubiquitinating enzyme(s).

Ke et al. (2008) showed that Ni can induce phosphorylation of histone H3 at its serine 10 (Ser10) residue in a c-jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK)-dependent manner. An inhibitor of JNK eliminated the Ni-initiated JNK-mediated induction of histone H3 phosphorylation at Ser10. A complete loss of Ni ion-induced phosphorylation of H3S10 was observed when JNK was specifically knocked down with RNAi.

Ji et al (2008) investigated epigenetic alterations in a set of DNA repair genes in NiS-transformed human bronchial epithelial (16HBE) cells. The silencing of the O(6)-methylguanine DNA methyltransferase (MGMT) gene locus and upregulation of DNMT1 expression was specifically detected in these cells. Moreover, epigenetic alterations including DNA hypermethylation, reduced histone H4 acetylation and a decrease in the ratio of Lys-9 acetylated/methylated histone H3 at the MGMT CpG island in NiS-transformed 16HBE cells were noted.

Arita et al. (2012) conducted a study in a Chinese population to determine whether occupational exposure to Ni is associated with alterations of global histone modification levels. Urinary Ni and global H3K4 trimethylation, H3K9 acetylation, and H3K9 dimethylation levels were measured in peripheral blood mononuclear cells of 45 subjects with occupational exposure to Ni and 75 referents.

H3K4me3 was significantly elevated in Ni-exposed subjects compared with referents, and H3K9me2 was decreased. H3K4me3 was positively and H3K9ac was negatively associated with urinary Ni.

7.5.3.3. Regulation of miRNAs

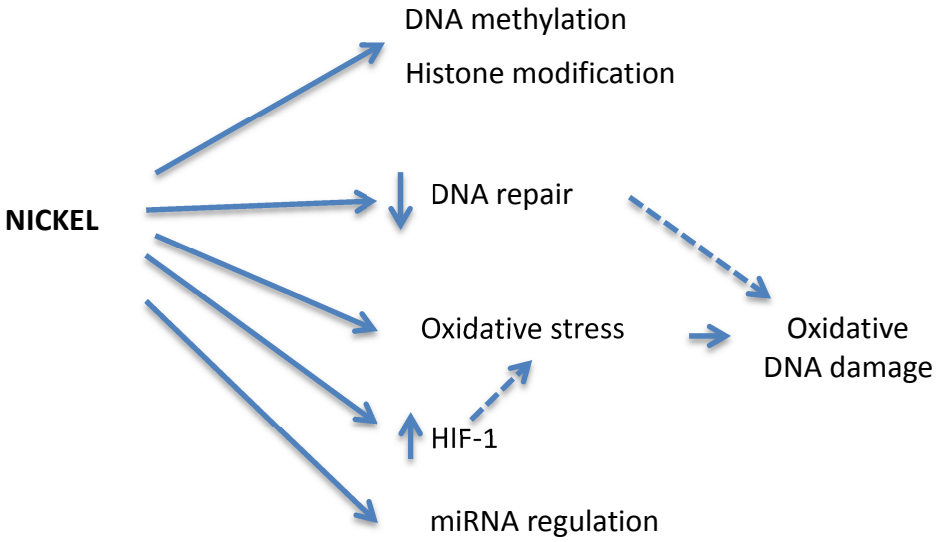
Recent studies have reported that miRNAs may play a role in Ni-induced cell transformation.

Zhang et al. (2013) reported that expression of miR-222 was significantly up-regulated in rat rhabdomyosarcomas induced by an intramuscular injection of Ni subsulphide as well as in Ni-transformed 16HBE cells. This miR is able to target several important tumour suppressor genes including *p27*, *p57* and *PTEN* thus contributing to accelerated cell growth observed in Ni-induced tumours as well as transformed cells.

miR-152, a tumour suppressor microRNA targeting DNMT1, was significantly down-regulated in Ni sulphide-transformed 16HBE cells (Ji et al. , 2013). Consequently, DNMT1 levels increased and led to elevated DNA methylation levels and enriched MeCP2 at the promoter of miR-152. Moreover, while ectopic expression of miR-152 in Ni sulphide-transformed cells inhibited cell proliferation, expressing anti-miR-152 in normal 16HBE cells resulted in increased cell proliferation and colony formation.

Zhang et al. (2013) investigated the expression of several miRNAs in Ni_3S_2-transformed 16HBE cells (NSTCs) and observed a strong downregulation of miR-203. Hypermethylation of CpGs in miR-203 promoter and first exon area was also detected, and proved to be involved in the Ni-induced cell transformation. miR-203 was able to suppress cell transformation at least in part through negatively regulating its target gene ABL1.

The complexity of the mechanisms of Ni genotoxicity including epigenetic modifications is represented in Figure 13.



HIF-1: hypoxia-inducible factor-1.

Figure 13: Mechanisms of Nickel genotoxicity and epigenetic mechanisms (modified from Henkler et al., 2010)

7.5.4. Sensitising activity of Nickel

Interactions of metal ions with proteins and the role for immune responses have been reviewed by Martin et al. (2006). There is evidence that combination of Ni with circulating or tissue protein gives rise to antigen specific responses, and thus Ni can act as contact allergen and cause sensitization. The antigens are taken up by antigen-presenting cells that migrate to draining lymph nodes, resulting in

activation of Ni-specific T lymphocytes. Contact sensitivity is either expressed as Type I or Type IV hypersensitivity, mediated by reagins and allergen-specific T lymphocytes, expressing in a wide range of cutaneous eruptions following dermal or systemic exposure. An alternative, but not mutually exclusive, hypothesis is that this metal interferes with the antigen recognition step of the immune response, i.e. binding to MHC and or MHC-bound peptides and T cell receptors leading to the activation of NI-specific T cells.

7.6. Dose-response assessment

7.6.1. Effects in experimental animals

The CONTAM Panel identified reproductive and developmental toxicity as the critical effect for the risk characterization of chronic oral exposure to Ni. Dose related effects have been reported in rodents for different reproductive (e.g. effects on male sex organ weights, histopathological changes in these organs, disturbed spermatogenesis, decreased sperm motility and sperm damages in mice) and developmental (e.g. increased pup mortality - stillbirth or post-implantation loss/perinatal lethality in rats) endpoints in a number of studies of varying size and quality, see Section 7.2.3. The most suitable and reliable dose-response information for reproductive and developmental effects were identified in a one-generation dose-range finding study (SLI, 2000a), denoted DRF, and a subsequent main 2-generation study (SLI, 2000b) denoted 2-GEN - see Section 7.2.3. The DRF study used five dose groups (2.2, 4.4, 6.6, 11, 17 mg Ni/kg b.w.) and a control group with 7–8 animals in each group. The 2-GEN study used four dose groups (0.2, 0.6, 1.1, 2.2 mg Ni/kg b.w) and a control group with 25–28 animals in each group. Since the developmental effects in the F2 generation of the main study were only investigated and reported in a small selected subset of animals, RPs derived from those data were used as supportive information only. For the dose-response assessment using the BMD approach, the CONTAM Panel identified the incidence of litters with post implantation loss per treatment group as a relevant and sensitive endpoint to assess dose-response of developmental toxicity of Ni in experimental animals. Although individual data of the post-implantation loss per litter (where each pup in each litter is characterized by the presence or absence of an effect occurring between implantation and birth) had been made available to EFSA through the confidential study reports (SLI, 2000a, b), these data were not chosen to derive a RP since the analysis of these nested dichotomous dose-response data using currently available software did not comply with the established goodness-of-fit criterion of EFSA (EFSA, 2009) and resulted in BMDL values depending strongly on the models used, see Appendix H2.2.

Both, the DRF and the 2-GEN study data were examined for presence of a dose-response relationship. Although there was a dose-dependent increase of the incidence of litters with post implantation loss per treatment group in both studies, a test for trend (Cochran-Armitage test) was not statistically significant ($P = 0.3$ and $P = 0.13$ for the DRF and the 2-GEN study, respectively, using the exact version). When performing the BMD analysis on both data sets and checking the loglikelihood criterion for the reduced, the full and the acceptable models the differences hardly indicated a clear dose-response relationship, although BMD/L values could be calculated (see Table 19 and for details Appendix H 2.1). Since both data sets were obtained under identical experimental conditions in the study of SLI (2000a, b) and since the BMD_{10} and $BMDL_{10}$ values of acceptable models were similar in the two data sets (minimum $BMDL_{10}$ of all acceptable models equal to 0.20 and 0.22 mg Ni/kg b.w. per day for the DRF and the 2-GEN study, respectively), the CONTAM Panel decided to derived a RP using the combined data on the incidence of litters with post-implantation loss per treatment group of the two studies. A statistically significant dose-response relationship ($P = 0.00013$ calculated with the Cochran-Armitage test for trend, exact version) and there was a statistically significant difference between the reduced and the full model and the acceptable models and the reduced model (based on the likelihood criterion) were observed, see Appendix H 2.1. Furthermore the combined data covered a dose range of two orders of magnitude and the admissible BMDLs (with ratio of BMD and BMDL not larger than one order of magnitude) were not smaller than the lowest dose tested, see Table H2.1 in the Appendix H2.

Therefore, the CONTAM Panel selected the $BMDL_{10}$ value of 0.28 mg Ni /kg b.w. per day as an RP for chronic exposure to Ni (see Table 19). The RP of 0.28 mg Ni /kg b.w. per day is supported by the BMD analysis of a related endpoint which accounted for the incidence of litters with three or more post-implantation losses - see Appendix H 2.1. The cut-off of 3 losses was selected on the basis of the mean values for post-implantation loss, calculated from the historical control groups of eight studies, which ranged from 0.88 to 2.31/litter, see Section 7.2.3. When analysing this endpoint in the same way as the overall incidence reported in Table 19, the $BMDL_{10}$ values ranged between 0.19 and 0.28 mg Ni/kg b.w. per day.

The Panel noted considerable model uncertainty due to the complexity of the developmental toxicity data and the choice of the critical endpoint.

Table 19: Results of the BMD analysis of the dose-response data on reproductive and developmental effects of nickel sulphate hexahydrate in rats observed for the F1 generation in the dose finding (DRF), the two-generation study (2-GEN) and the combined DRF and 2-GEN studies of SLI (2000 a, b), respectively

a) F1 generation in the dose finding study using 0, 2.2, 4.4, 6.6, 11, 17 mg Ni/kg b.w. per day

Data set	critical effect	model (specification)	Goodness-of-fit	BMD_{10}	$BMDL_{10}$
				(mg Ni/kg b.w. per day)	(mg Ni/kg b.w. per day)
F1 in DRF	incidence of litters with post-implantation per treatment group	multistage	0.89	0.48	0.20

b) F1 generation in the main (2-GEN) study using 0, 0.2, 0.6, 1.1, 2.2 mg Ni/kg b.w. per day

Data set	critical effect	model (specification)	Goodness-of-fit	BMD_{10}	$BMDL_{10}$
F1 in 2-GEN	incidence of litters with post-implantation per treatment group	multistage	0.45	0.72	0.22

c) F1 generation in the combined (DRF/2-GEN) study using 0, 0.2, 0.6, 1.1, 2.2, 4.4, 6.6, 11, 17 mg Ni/kg b.w. per day

Data set	critical effect	model (specification)	Goodness-of-fit	BMD_{10}	$BMDL_{10}$
F1 in DRF+2-GEN combined	incidence of litters with post-implantation per treatment group	multistage	0.54	0.76	0.28

2-GEN: 2-generation; b.w.: body weight.

Two other studies on reproductive and developmental toxicity were identified which reported dose-response data suitable for a BMD analysis (Smith et al., 1993; Panday and Srivastava, 2000). The study on reproductive toxicity of Panday and Srivastava (2000) reported a dose response relationship for the percentage of motile sperms in epididymis (in units of 10^{-7}) and percentage of abnormal sperms in Swiss albino mice treated for 35 days (five days per week) with Ni sulphate or Ni chloride and a joint control group - see Section 7.2.3.

The PROAST software (version 26) was applied for continuous data as described above. Using the BMR of 5 %, the $BMDL_{05}$ for the best fitting models was calculated as 0.42 and 0.46 mg Ni (sulphate)/kg b.w. per day and 0.38 and 0.43 mg Ni (chloride)/kg b.w. per day for percentage of motile sperms and sperm count in epididymis, respectively - see Appendix G. No complete BMD analysis was possible for percentage of abnormal sperms since explicit data on the variability of the estimates were missing.

Smith et al. (1993) studied reproductive and developmental toxicity in female Long-Evans rats treated with Ni chloride in two breedings - see Section 7.2.3. Whereas the numbers of alive and dead pups showed no dose-response relationship a dose dependent trend was observed for the number of litters with dead pups at birth and the mean values and the percentage of dead pups at PND1 and PND21. Since mean values do not account for individual litter size and since no information on the inter-individual variability had been reported a dose-response analysis using the BMD approach was only performed on the number of litters with dead pups at birth. Therefore, the CONTAM Panel applied the BMD approach for quantal (dichotomous) data as described above. Using the default BMR of 10 % extra risk a $BMDL_{10}$ of 1.6 mg Ni/kg b.w. per day was calculated for the data from the 2nd breeding - see Appendix G. For the 1st breeding none of the models available in BMDS were acceptable according to EFSA practices (EFSA 2009, 2011e). It was also noted that the estimated effective dose for both breedings varied over the treatment phase and between animals such that the $BMDL_{10}$ obtained from this study would carry substantial uncertainty. Due to the limitations in design and data reporting, the CONTAM Panel did not use the results of these studies for deriving an RP for the chronic exposure of experimental animals but noted that the BMDLs calculated from these data would not contradict with the $BMDL_{10}$ of 0.28 mg Ni /kg b.w. derived from the studies of SLI (2000, a, b) which were of much higher quality.

7.6.2. Effects in sensitized humans

Systemic contact dermatitis elicited in Ni-sensitive humans after oral exposure seen as flare-up reactions, worsening of allergic reactions (e.g. hand eczema, body erythema) were analysed by Jensen et al. (2006) in a 'modified meta-analysis' of 17 studies published between 1979 and 2003 on a total of about 450 patients. The authors calculated effective doses (ED) corresponding to selected response rates ranging from 1 % to 50 %. ED_{10} values for 10 % response, which is in line with the default BMR for quantal data, were 0.55 , 0.87 and 1.33 mg Ni per person for the high, intermediate and low Ni sensitivity groups, with corresponding 95 % confidence intervals of 0.17–0.86, 0.31–1.26 and 0.53–1.94, respectively. Assuming 70 kg as default adult b.w., a lower bound of the ED_{10} defined in such a manner could be as low as 2.4 µg Ni/kg b.w. per day for acute exposure. The CONTAM Panel noted that this meta-analysis was not suitable for deriving a health-based guidance value (see Section 7.3.2). For this reason, the CONTAM Panel decided not to use the ED_{10} values or their lower confidence limits calculated by Jensen et al. (2006) for risk characterization except as supporting information.

Three studies (Gawkrodger et al., 1986; Hindsén et al., 2001; and Jensen et al., 2003, see Section 7.3.2) among those used in the meta-analysis of Jensen et al. (2006) were identified as suitable for dose-response analysis and the CONTAM Panel performed a BMD analysis on these. No other studies since the publication of Jensen et al. (2006) exhibiting dose-response data of similar or better quality could be identified in the literature. When applying the BMD approach for quantal data, as described in Section 7.6.1 above, to the data of the three studies, the CONTAM Panel identified the data of Jensen et al. (2003), with incidences of 1/10, 4/10, 4/10 and 7/10 at the doses 0, 0.3, 1, and 4 mg Ni per person, respectively as the most sensitive and from derived a $BMDL_{10}$ of 0.08 mg Ni per person, corresponding to 1.1 µg Ni/kg b.w., as an RP for systemic contact dermatitis elicited in Ni-sensitive humans after acute oral exposure to Ni, see Table 20. The CONTAM Panel noted that this value of 1.1 µg Ni/ kg b.w is in the same range as the lower confidence bounds of the ED_{10} values calculated in the meta-analysis by Jensen at al. (2006).

Table 20: Result of the BMD analysis on the incidence of systemic contact dermatitis after exposing sensitized humans observed in three studies selected to assess acute exposure of humans assuming a body weight of 70 kg

Source of dose-response data	BMD_{10} µg Ni/kg b.w.	$BMDL_{10}$ µg Ni/kg b.w.
Gawkrodger et al. (1986)	5.8	2.6
Hindsén et al. (2001)	2.6	1.6
Jensen et al. (2003)	2.6	1.1

7.7. Derivation of health-based guidance value/margin of exposure

The CONTAM Panel considered the critical effects of Ni in order to derive health based guidance values (HBGV).

7.7.1. Chronic effects

The CONTAM Panel selected the effects on reproduction and development as the critical effects for establishing a chronic health- based guidance value for Ni. In particular, the increased incidence of litters with post-implantation loss observed in different reproductive toxicity studies in rats was identified as the critical effect, and a $BMDL_{10}$ of 0.28 mg/kg b.w. per day, calculated from the dose-response analysis of the combined data of a 1-generation dose range finding study and a 2-GEN study in rats, was selected as the chronic RP (see Section 7.6.1). This RP is lower than that derived by other institutional bodies using the same studies (NOAEL of either 1.1 or 2.2 mg/kg b.w. per day, see Section 1.3) but it should be considered that this is the first time a dose-response analysis of the complete data sets of these studies using the BMD approach is applied. As concluded by the EFSA's Scientific Committee (EFSA, 2009), the BMD approach is a scientifically more advanced method to the NOAEL approach for deriving a RP, since it makes extended use of available dose-response data and it provides a quantification of the uncertainties in the dose-response data. Other studies were modelled to be considered as supporting information. In particular, the CONTAM Panel identified two studies in mice indicating adverse effects on male fertility at low doses. While, due to methodological limitations, these studies were considered not adequate for the hazard characterisation, a tentative dose response analysis on sperm motility and sperm count indicated that the $BMDL_{05}$ values calculated for those quantitative data were in the range 0.38–0.46 mg Ni/kg b.w. per day (see Section 7.6.1), supporting the selected RP of 0.28 mg Ni/kg b.w. per day. From the selected $BMDL_{10}$ of 0.28 mg Ni/kg b.w. per day the CONTAM Panel derived a TDI of 2.8 µg Ni/kg b.w. per day by applying the default uncertainty factor of 100 to account for extrapolation from experimental animals to humans and for inter-individual variability.

7.7.2. Hypersensitivity reactions

Allergic contact dermatitis is the most prevalent effect of Ni in the general population. It has been reported that individuals sensitised to Ni through dermal contact and who have allergic contact dermatitis (estimated prevalence in the general population to be up to 15 %, but frequently remaining undiagnosed) may develop hand eczema from oral exposure to Ni salts. The TDI of 2.8 µg Ni/kg b.w. per day may therefore not be sufficiently protective of individuals sensitized to Ni. The CONTAM Panel identified three studies addressing this issue which were suitable for dose-response analysis of acute oral exposure to Ni in sensitised humans and performed a BMD analysis. As a result, $BMDL_{10}$s in the range 1.1–2.6 µg Ni/kg b.w. per day were calculated (see Section 7.6.2). The Panel selected the lowest $BMDL_{10}$ of 1.1 µg Ni/kg b.w. as RP for acute oral exposure to Ni.

Dose-dependent relationships between the amount of Ni ingested and the probability of a dermatitis flare and between the amount ingested and the severity of flares has been demonstrated in several studies (Jensen et al., 2006). It is generally accepted amongst scientists in the field of immunotoxicology and sensitization that contact sensitization as well as elicitation of responses in sensitized individuals follow dose response relationships and have a threshold (Friedman, 2007;

Kimber and Basketter, 2008). This is also true for hypersensitivity to Ni (Ross-Hansen et al., 2014). For Ni ingested via the oral route, this implies that access of Ni molecules to the skin may lead to hypersensitivity reactions in the skin in a dose-dependent fashion.

On the other hand, thresholds have not been formally established for sensitization to most contact allergens, and information on thresholds of allergic reactions in sensitized individuals is even sparser. On the basis of these considerations the CONTAM Panel decided not to define an acute reference dose, but to adopt a margin of exposure (MOE) approach for risk characterization of this critical effect. The selected RP is based on a highly sensitive study group (sensitized individuals) of fasted individuals given Ni sulphate in lactose capsules. Under these conditions, absorption is assumed to be considerably higher than from food. These considerations suggest that the selected RP could be conservative for the characterisation of the acute risks. It should be noted that the reported absorption of Ni ranges from 1 to 40 % and it is particularly low when exposure occurs in the presence of food or under non-fasted conditions (see Section 7.1). Moreover, the critical effect, i.e. hand dermatitis or generalized eczematous flare-up reactions, is considered relatively less severe as compared to other toxic effects in humans. On the other hand, the CONTAM Panel took into account the large inter-individual variability in the immune response that might not be covered by the limited number of individuals examined in the selected studies. Therefore the CONTAM Panel decided that an MOE of 10 or higher would be indicative of a low health concern.

8. Risk characterisation

8.1. Chronic effects

The CONTAM Panel established a TDI of 2.8 µg/kg b.w. per day for Ni. The mean chronic dietary exposure to Ni, across the different dietary surveys and age classes, ranging from 2.0 (minimum LB, 'Elderly') to 13.1 µg Ni/kg b.w. per day (maximum UB, 'Toddlers') is close to the TDI or above it particularly when considering the young age classes (e.g. 'Infants', 'Other children', 'Toddlers' and 'Adolescents'). The 95th percentile dietary exposure ranging from 3.6 (minimum LB, 'Elderly') to 20.1 µg Ni/kg b.w. per day (maximum UB, 'Toddlers') is above the TDI for all age classes. Therefore, the CONTAM Panel concluded that the current dietary exposure to Ni is of concern for the general population.

Regarding the vegetarian population, although based on limited consumption data, the dietary exposure to Ni seems to be slightly higher than in the general population analysed in the same dietary survey (see Table 10), with a highest estimated 95th percentile exposure of 7.1 µg Ni/kg b.w. per day. Therefore, the level of concern for dietary exposure to Ni for the general population can be extended to the vegetarian population.

8.2. Acute effects

For the acute effects, an acute RP of 1.1 µg Ni/kg b.w. for hypersensitivity reactions was established for an MOE approach. The estimated mean dietary acute exposure in the young population ('Infants', 'Toddlers', 'Other children', and 'Adolescents') ranged from 3.4 (95 % CI = 3.1-3.7) µg/kg b.w. to 14.3 (95 % CI = 13.2-15.5) µg/kg b.w. In the adult population ('Adults', 'Elderly', and 'Very elderly'), the mean dietary acute exposure ranged from 2.5 (95 % CI = 2.2–2.9) µg/kg b.w. to 4.9 (95 % CI = 4.6–5.5) µg/kg b.w. The 95th percentile ranged from 8.6 (95 % CI = 8.0–9.1) µg/kg b.w. to 35.0 (95 % CI = 26.8–47.2) µg/kg b.w.in the young population, and from 5.5 (95 % CI = 5.1–6.0) µg/kg b.w. to 11.8 (95 % CI = 10.6 -13.8) µg/kg b.w. in the adult population.

All the MOEs calculated from these exposure levels are considerably below 10 for all age groups both for the estimated mean and 95th percentile exposure levels.

As indicated earlier, the RP was based on a highly sensitive population (sensitized individuals) examined under fasting conditions, in which the Ni absorption is significantly higher than via food. Out of the human volunteer studies suitable for the dose response analysis, the CONTAM selected the

study by Jensen et al. (2003) resulting in the lowest $BMDL_{10}$ for the derivation of the acute RP. Finally, even in this study, at the intake levels that still produced eczematous flare-up reactions in sensitized individuals, not all individuals in fact developed such reactions. Taking into account these elements in the MOE interpretation, it cannot be predicted that all sensitized individuals will actually develop adverse reactions, nor what percentage eventually will develop such reactions at the estimated levels of Ni intake. Overall, the CONTAM Panel concluded that, at the current levels of acute dietary exposure to Ni, there is a concern that Ni-sensitized individuals may develop eczematous flare-up skin reactions.

9. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to Ni in food and drinking water has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006), the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters).

9.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference.

9.2. Exposure scenario/Exposure model

In response to EFSA's request to submit occurrence data on Ni in food and drinking water, 57 928 samples were reported in the EFSA database. After the quality assessment of the reported data, 44 585 samples were available for exposure calculations, among them 25 700 for drinking water. Around 35 % of the analytical results for food and 90 % for drinking water were left-censored. All food groups (FoodEx level 1) were well represented, with 'Grain and and grain-based products' and 'Vegetable and vegetable products' reporting the highest number of samples with 4 291 and 3 738, respectively. The majority of the drinking water samples belonged to 'Tap water' (73 %). The samples were collected mostly by one Member State. Therefore, there is an uncertainty from possible regional differences in the presence of Ni in food commodities and drinking water, and it is evident that the dataset is not fully representative for all Member States and the EU.

Food preparation using stainless steel containers, processors and utensils may contribute to the concentration of Ni present in food, particularly in food contact materials made of poor quality stainless steel, or of other metal alloys containing Ni. Since occurrence data on food as consumed were practically not present in the dataset used, this might have led to an underestimation of the chronic and the acute exposure to Ni in food.

A large proportion of samples with left-censored data introduce considerable uncertainties to the overall dietary exposure estimate. The LB values reported in this opinion tend to underestimate, while the UB values tend to overestimate the chronic and the acute dietary exposure to Ni in food. Relatively low differences were observed between the exposure estimations at the LB and UB scenarios in this opinion.

The average contribution of 'Alcoholic beverages' to the dietary exposure to Ni was overall negligible. The median average contribution across dietary surveys for adults, elderly and very elderly ranged between 0.8 % (LB) and 2.1 % (UB). Several samples of beers with unusual high levels of Ni (> 5 mg/kg, range 5 200–14 300 µg/L) were reported in the original dataset. Different hypotheses can be formulated about the origin of such high values, from the water used in the elaboration to contact materials used during the brewing process, the presence of Ni in the cereals used or the use of some old-fashioned practices to increase stability of beer foam through addition of Ni (Rudin, 1957; Hudson, 1959; Luykx, 1960). Data providers were contacted to confirm these values and to gather further information on the samples. Since no answer was received from the data providers, the

CONTAM Panel excluded these data from the final dataset (see Section 4.2.1.). It is important to note that the inclusion of these samples would have implied an increase of exposure to Ni by up to 2–3 times in certain dietary surveys based on the beer consumption habits, and 'Alcoholic beverages' to became the main contributor to the dietary exposure to Ni. Therefore, there is some uncertainty in the potential contribution that beer may have in the dietary exposure to Ni.

There is also uncertainty associated to the contribution of the food group 'Legumes, nuts and oilseeds' to the total exposure to Ni since the distinction between raw and cooked/consumed food is not always clear in the dietary surveys. Some overestimation of the dietary exposure to Ni and in the average contribution of this food group may not be discarded.

There is uncertainty associated to the dietary exposure calculated for the vegetarian population since very limited consumption data in this population are available. There are also insufficient data on consumption for children younger than one year (infants), which adds uncertainty to the exposure calculations in this age group.

Overall, the CONTAM Panel noted that there is considerable uncertainty regarding the total dietary exposure to Ni from food and drinking water.

9.3. Model input (parameters)

One European standardized method exists for the determination of Ni in food but only for animal and vegetable fats and oils, in contrast to the existence of four standardized methods for Ni in drinking water.

Several standard or certified reference materials are available and a number of proficiency testings are regularly organized for the measurement of Ni in food and drinking water.

The analytical results used for exposure assessment were performed by different laboratories at largely varying LOQ/LODs. Those limitations may have added to the overall uncertainty of the analytical results

9.4. Other uncertainties

Several kinetic studies in humans and experimental animals indicate that oral absorption of soluble nickel species is more efficient when administered in drinking water or other beverages under fasting conditions, than via solid food. There is uncertainty in the systemic absorption rate of the key studies identified for the derivation of the acute and chronic RPs, in which Ni was administered via gavage using an aqueous solution as vehicle in the rat, or via lactose capsules under fasting conditions in human volunteer studies. Furthermore, the study used for the acute RP derivation did not consider the contribution of the dietary exposure in the estimation of the Ni doses tested in human volunteers. Overall the CONTAM Panel noted that the use of external exposure levels not taking into account the differences in bioavailability adds considerably to the uncertainty of the assessment, and it is possibly associated to an overestimation of the risk. The CONTAM Panel considered it appropriate to establish a TDI for chronic exposure to Ni based on the $BMDL_{10}$ values for reproductive and developmental toxicity based on data from a well conducted multi-generation study in rats using of post-implantation loss in the F0/F1 generation per litter as the most suitable endpoint. It was noted that this endpoint could be analysed using aggregate data such as the incidence of litters with post- implantation loss per treatment group or using the raw individual data of the offspring (presence or absence of an effect occurring between implantation and birth). Statistical modelling for the latter data is more complex and less developed and the application of available models did not result in sufficient goodness-of-fit when using the criteria established in EFSA (2009) and such the BMD/L values derived from these hierarchical/nested data (appearing higher than those obtained from the aggregated data, see Appendix H) were not used for risk characterization. This adds to the uncertainty of the RP used for risk characterization. Furthermore, availability of relevant dose-response data for only one species of experimental animals (e.g. missing data on the fertility and development in mice) adds also to the

uncertainty. Observations in humans showed toxicity of Ni in humans at very high doses resulting after accidental or intended oral, occupational or other intoxication. However, epidemiological data from well conducted studies on human dietary exposure to Ni have been rare and were negative or inconclusive. This is in contrast to studies on humans who were primarily exposed to Ni via inhalation during occupation which could be used to classify Ni as carcinogenic to humans causing cancer of the lung and nasal cavity.

9.5. Summary of uncertainties

Summaries of the uncertainty evaluations for Ni, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source might have led to an over- or underestimation of the exposure or the resulting risk, are presented in Table 21.

Table 21: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to Ni in food and drinking water

Sources of uncertainty	Direction[(a)]
Measurement uncertainty of analytical results	+/-
Extrapolation of occurrence data to the whole of Europe	+/-
Use of LB and UB occurrence data in the dietary exposure estimations	+/-
Possible use of occurrence data from targeted sampling	+
Linkage between the occurrence data in raw food and the consumption data on food as consumed	+
Insufficient data on the presence of Ni in some foods such as beverages including beer	-
Insufficient data on the impact of exposure from smoking to the dietary exposure	-
Limited data on exposure for specific groups (vegetarians)	+/-
Limited data on exposure from human milk based on limited data	+/-
Limited information on exposure of infants	+/-
Non-consideration of the potential migration of nickel from food contact material during food preparation	-
Different absorption rates of soluble nickel via ingestion of drinking water and beverages under fasting conditions and via solid food	+
Selection of the most relevant reproductive toxicity endpoint for use in the dose response modelling	+/-
Quality of available studies on fertility and human relevance of the fertility effects observed in experimental animals	+/-

(a): +: uncertainty with potential to cause over-estimation of exposure/risk; -: uncertainty with potential to cause under-estimation of exposure/risk

Overall, the CONTAM Panel concluded that the impact of the uncertainties on the risk assessment of exposure to Ni in food is large and the risk assessment is more likely to overestimate than to underestimate the risks.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Nickel (Ni) is a widespread component of Earth's surface: it is found in all environmental compartments and is ubiquitous in the biosphere. Its presence in food and drinking water can arise from both natural and anthropogenic sources.

- Ni can exist in different oxidation states; however, in food and drinking water Ni generally occurs in the divalent form - Ni^{2+} or Ni(II) - its most stable oxidation state.

- Ni has a very wide range of industrial/commercial uses. In particular, it may be present in inexpensive alloys for fashion or junk jewellery and, as a Ni flash, in the silver or gold plating process of the aforesaid jewellery.

Sampling and methods of analysis

- Four European standardised methods for the determination of total Ni in water are available while only one standardised method is available for food, only in animal and vegetable fats and oils.

- Several analytical techniques are suitable for the determination of total Ni in foods and waters. Furnace or graphite furnace with atomic absorption spectrometry, and increasingly inductively coupled plasma-optical atomic emission or inductively coupled plasma-mass spectrometry (ICP-MS) have been used.

- Several standard or certified reference materials are available for total Ni in food and water.

- Regular proficiency testing schemes are organised by a number of providers for total Ni in food and water.

Occurrence

- A total of 18 885 food samples and 25 700 drinking water samples were available in the final dataset to estimate dietary exposure to Ni. No speciation data were provided.

- Samples were collected between 2003 and 2012 in 15 different European countries, with almost 80 % of the total collected in one Member State.

- The most reported analytical methods were inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectrometry, that represented 54 % and 42 % of the methods reported, respectively.

- In the final dataset, left-censored data represented 66 % of the analytical results, with 35 % in food samples and 89 % in drinking water samples.

- At FoodEx level 1, all food groups were well represented, with a maximum of 25 700 samples of 'Drinking water' and 4 291 and 3 738 samples in the food groups 'Grain and grain-based products' and 'Vegetables and vegetable products (including fungi)', respectively.

- High mean levels of Ni were reported for 'Legumes, nuts and oilseeds' (~ 2 mg/kg), certain types of chocolate (cocoa) products (3.8 mg/kg), and 'Cocoa beans and cocoa products' (9.5 mg/kg).

- The potential leaching of Ni into food from food contact material is not covered by the occurrence dataset used to estimate dietary exposure.

Exposure to nickel via food and drinking water

Chronic exposure

- Mean chronic dietary exposure to Ni, across the different dietary surveys and age classes, ranged from 2.0 (minimum lower bound (LB), 'Elderly') to 13.1 µg/kg body weight (b.w.) per day (maximum upper bound (UB), 'Toddlers'). The 95th percentile dietary exposure ranged from 3.6 (minimum LB, 'Elderly') to 20.1 µg/kg b.w. per day (maximum UB, 'Toddlers').

- Among the different age classes, 'Toddlers' and 'Other children' showed the highest chronic dietary exposure to Ni.

- Overall, the main contributors to the dietary exposure to Ni across the different dietary surveys and age classes were 'Grain and grain-based products', 'Non alcoholic beverages (except milk-based beverages)', 'Sugar and confectionery', 'Legumes, nuts and oilseeds', and 'Vegetables and vegetable products (including fungi)'. 'Milk and dairy products' were also important contributors to the dietary exposure to Ni in the young population, in particular in toddlers. The contribution of 'Drinking water' to the total exposure to Ni was very small across dietary surveys and age classes (0.0005 %–1.7 %, LB-UB).

- Although based on very limited consumption data, both average and highly exposed vegetarian population seem to have slightly higher dietary exposure to Ni than the general population.

Acute exposure

- Highest levels for acute dietary exposure were observed in toddlers and other children.

- Mean dietary acute exposure in the young population ('Infants', 'Toddlers', 'Other children' and 'Adolescents') ranged from 3.4 (95 % confidence interval (CI) = 3.1–3.7) µg/kg b.w. in one survey for 'Adolescents' to 14.3 (95 % CI = 13.2-15.5) µg/kg b.w.in one survey for 'Toddlers'. The 95th percentile ranged from 8.6 (95 % CI = 8.0–9.1) µg/kg b.w. in one survey for 'Adolescents' to 35.0 (95 % CI = 26.8–47.2) µg/kg b.w. in one survey for 'Toddlers'.

- Mean dietary acute exposure in the adult population ('Adults', 'Elderly' and 'Very elderly') ranged from 2.5 (95 % CI = 2.2–2.9) µg/kg b.w. in one survey for 'Elderly' to 4.9 (95 % CI = 4.6–5.5) µg/kg b.w. in one survey for 'Adults'. The 95th percentile ranged from 5.5 (95 % CI = 5.1–6.0) µg/kg b.w. in one survey for 'Elderly' to 11.8 (95 % CI = 10.6–13.8) µg/kg b.w. in one survey for 'Adults'.

Non dietary exposure

- Both for smokers and non-smokers not-occupationally exposed to Ni, exposure by inhalation may be expected in general to represent a negligible or minor addition to the daily exposure via the diet.

Hazard identification and characterisation

Toxicokinetics

- Following oral exposure, Ni is bioavailable at levels from as low as 1 % up to 40 % in humans, with a lower bioavailability when exposure occurs in the presence of food than when Ni is dosed in drinking water alone.

- The absorbed Ni can bind to serum proteins and widely distribute in the organism. Ni is actively transferred across the blood-placental barrier into the fetus. Absorbed Ni is excreted mainly via the urine and, to a lower extent in breast milk. An estimated elimination half life of 28 ± 9 hours was calculated in human volunteers.

Repeat dose toxicity

- Major effects observed in repeated dose toxicity studies in rats were decreases in b.w., effects on organ weights (liver and kidneys), hepatotoxicity, nephrotoxicity, and irritation of gastrointestinal tract at high doses.

- The primary toxic effects observed in mice were in the myeloid system.

Developmental and reproductive toxicity

- In rats, oral administration of Ni compounds does not induce alterations in reproductive tissues and no adverse effects on fertility or reproductive performances were reported.

- In mice, effects on male sex organs weights, histopathological changes in these organs, disturbed spermatogenesis, decreased sperm motility and sperm damages have been reported in studies after oral exposure to Ni compounds and were responsible for a decrease in fertility. Limitations in these studies preclude their use for the establishment of a Reference Point (RP).

- There is consistent evidence of increased pup mortality (stillbirth or post-implantation/perinatal lethality) after exposure of rats to Ni chloride or sulphate in several reproductive toxicity studies.

- In mice exposed to Ni chloride, malformations, reduced ossification and increased incidence of skeletal anomalies were observed at doses ≥ 92 mg Ni/kg b.w. per day in the presence of maternal toxicity. Microphthalmia was observed at 46 mg Ni/kg b.w. per day in the absence of maternal toxicity.

- Ni is considered to be a developmental toxicant inducing fetotoxicity, embryotoxicity and teratogenicity.

Genotoxicity

- Soluble Ni compounds are not mutagenic in bacterial cells and, in general, weakly mutagenic in mammalian cells *in vitro*.

- Chromosomal effects due to both aneugenic and clastogenic activity of soluble Ni compounds have been observed in mammalian cells *in vitro*. The evidence for *in vivo* induction of chromosomal alterations is inconsistent

- There is evidence for the induction of DNA damage by soluble Ni compounds both *in vitro* and *in vivo*.

Carcinogenicity

- Ni compounds have been shown to induce tumours in experimental animals by inhalation and injection at several different sites.

- No tumours were found in animals that received soluble Ni compounds by the oral route.

Human observations

- The general population is primarily exposed to Ni via food and drinking water, whereas inhalation from ambient air and percutaneous absorption are generally minor sources of exposure.

- Subpopulations of possibly higher exposure are workers in Ni producing and related industries when exposed to airborne fumes, dusts and mists, and smokers.

- Ni and Ni compounds have been classified by IARC (2012) as human carcinogens causing cancers of the lung, nasal cavity and paranasal sinuses after inhalation. Based on i) the lack of epidemiological data suggesting that Ni compounds cause cancer at additional sites or by

additional routes, ii) the lack of tumours in the oral carcinogenicity studies in experimental animals and, iii) the modes of action, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) considered it unlikely that dietary exposure to Ni results in cancer in humans.

- Non-carcinogenic health effects of oral exposure to Ni include effects on the gastrointestinal, hematological, neurological and immune system.

- Gastrointestinal (vomiting, cramps, and diarrhea) and neurological symptoms (giddiness, headache, and weariness) were the most reported effects after acute exposure.

- The currently available epidemiological data do not support the existence of an association between dietary exposure to Ni and reproductive and developmental effects in humans.

- Exposure through skin or by inhalation may lead to Ni sensitization.

- Whereas oral exposure to Ni is not known to lead to sensitization, oral absorption of Ni is able to elicit eczematous flare-up reactions in the skin in Ni-sensitized individuals (systemic contact dermatitis).

Biomonitoring

- In subjects exposed to the same species of Ni from the same absorption route, Ni concentrations in serum and especially in urine are useful biomarkers of exposure and can be used for bio-monitoring purposes. However, due to inter- and intra-individual variability, from a single point estimate it is impossible to back-calculate the contribution of intake from food to Ni concentration in accessible biological media.

Modes of action

- Ni can cross-link aminoacids to DNA, lead to formation of reactive oxygen species (ROS), and moreover mimic hypoxia. These changes may lead to the activation of some signalling pathways, subsequent transcription factors and eventually to alterations in gene expression and cellular metabolism.

- Ni^{2+} exerts effects directly on the developing embryo/fetus (crossing the placenta), as well as indirectly by altering the maternal hormonal balance.

- The mechanism of genotoxicity of soluble Ni compounds includes interference with cellular oxido-reductive regulation and induction of oxidative stress, inhibition of DNA repair systems, dysregulation of signalling pathways and alteration of the epigenetic landscape.

- Combination of Ni with circulating or tissue protein gives rise to new antigens and act as contact allergen and cause sensitization. Alternatively, binding to MHC and or MHC-bound peptides and T cell receptors leading to the activation of Ni-specific T cells may result in sensitization.

Dose response analysis

Chronic effects

- The CONTAM Panel identified reproductive and developmental toxicity as the critical effect for the risk characterization of chronic oral exposure to Ni.

- The Panel used the combined data from a dose range finding 1-generation study and a subsequent full 2-generation study in rats and derived a lower 95 % confidence limit for a benchmark response at 10 % extra risk ($BMDL_{10}$) value of 0.28 mg Ni /kg b.w. per day for post-implantation loss as RP for chronic dietary exposure to Ni.

Acute effects

- Systemic contact dermatitis elicited in Ni-sensitive humans after oral exposure seen as eczematous flare-up reactions and worsening of allergic reactions (e.g. hand eczema, body erythema) were identified as the critical effect for acute oral exposure to Ni of Ni-sensitized humans.

- Three data sets on sensitized human individuals with a history of contact dermatitis or related symptoms exposed orally to Ni in clinical challenge studies were identified as suitable for dose-response analysis using the benchmark dose (BMD) approach.

- When applying the BMD approach for quantal data a $BMDL_{10}$ of 1.1 µg Ni/ kg b.w. was calculated as an RP for eliciting systemic contact dermatitis in Ni-sensitive humans after acute oral exposure to Ni.

Derivation of Health-Based Guidance Value/Margin of exposure approach

Chronic effects

- The Panel derived a tolerable daily intake (TDI) of 2.8 µg Ni/kg b.w. from a $BMDL_{10}$ of 0.28 mg Ni/kg b.w. as calculated from the dose response analysis of the incidence of post-implantation loss in rats, applying the default uncertainty factor of 100 to allow for interspecies differences and human variability.

Acute effects

- The CONTAM Panel used the selected acute RP of 1.1 µg Ni/kg b.w. in a margon of exposure (MOE) approach for risk characterization.

- This selected RP is calculated on data obtained in a highly sensitive study group of fasted individuals given Ni sulphate in lactose capsules. Under these conditions, absorption is assumed to be considerably higher than from food. These considerations suggest that the selected RP could be conservative for the characterisation of the acute risks.

- On the other hand, the CONTAM Panel took into account the large inter-individual variability in the immune response that might not be covered by the limited number of individuals examined in the selected studies.

- Overall, the CONTAM Panel decided that a MOE of 10 or higher would be indicative of a low health concern.

Risk characterisation

Chronic effects

- The mean chronic dietary exposure to Ni, across the different dietary surveys and age classes, ranging from 2.0 (minimum LB, 'Elderly') to 13.1 µg Ni/kg b.w. per day (maximum UB, 'toddlers') is close to the TDI or above it, particularly when considering the young population ('Infants', 'Other children', 'Toddlers' and 'Adolescents').

- The 95th percentile dietary exposure ranging from 3.6 (minimum LB, 'Elderly') to 20.1 µg Ni/kg b.w. per day (maximum UB, 'Toddlers') is above the TDI for all age classes.

- The current dietary exposure to Ni raises concern when considering the mean and 95th percentile chronic exposure levels for all age classes.

- Although based on limited consumption data, the dietary exposure to Ni of the vegetarian population seems to be slightly higher than that estimated for the general population, with a highest estimated 95th percentile exposure of 7.1 µg Ni/kg b.w. per day. Therefore, the level of concern for dietary exposure to Ni for the general population can be extended to the vegetarian population.

Acute effects

- The MOEs calculated considering the estimated mean and the 95th percentile acute exposure levels and the acute RP of 1.1 µg Ni/kg b.w. were considerably below 10 for all age classes.

- Due to the approach followed for the derivation of the acute RP, it cannot be predicted that all sensitized individuals will actually develop adverse reactions, nor what percentage eventually will develop such reactions at the estimated levels of Ni intake.

- Overall, the CONTAM Panel concluded that, at the current levels of acute dietary exposure to Ni, there is a concern that Ni-sensitized individuals may develop eczematous flare-up skin reactions.

RECOMMENDATIONS

- There is a need for mechanistic studies to assess the human relevance of the effects on reproduction and development observed in experimental animals.

- There is a need for additional studies on human absorption of Ni from food, for example in combination with duplicate diet studies.

DOCUMENTATION PROVIDED TO EFSA

The following unpublished studies were received from US EPA:

1. RTI, 1985. Final report (draft): Dose-range finding study of nickel chloride administered to CD rats in the drinking water. Research Triangle Park, NC: Office of Solid Waste Management, US Environmental Protection Agency.

2. RTI, 1986. Two-generation reproduction and fertility study of nickel chloride administered to CD rats in the drinking water: 90-Day exposure of CD rats to nickel chloride administered in the drinking water. Final study report (I of III). Research Triangle Park, NC: Office of Solid Waste Management, US Environmental Protection Agency.

3. RTI, 1988a. Two-generation reproduction and fertility study of nickel chloride administered to CD rats in the drinking water: Fertility and reproductive performance of the P generation. Final study report (II of III). Research Triangle Park, NC: Office of Solid Waste Management, US Environmental Protection Agency.

4. RTI, 1988b. Two-generation reproduction and fertility study of nickel chloride administered to CD rats in the drinking water: Fertility and reproductive performance of the F1 generation. Final study report (III of III). Research Triangle Park, NC: Office of Solid Waste Management, US Environmental Protection Agency.

5. American Biogenics Corporation, 1988. Ninety day gavage study in albino rats using nickel. Draft Final Report submitted to Research Triangle Institute, Research Triangle Park, NC.

Unpublished studies received from the Nickel Institute:

1. SLI, Springborn Laboratories. 2000a. A one-generation reproduction range-finding study in rats with nickel sulfate hexahydrate. Spencerville, OH: Springborn Laboratories, Inc. SLI Study No. 3472.3.

2. SLI, Springborn Laboratories. 2000b. An oral (gavage) two-generation reproduction toxicity study in Sprague-Dawley rats with nickel sulfate hexahydrate. Final Report. Volume 1 of 3. Spencerville, OH: Springborn Laboratories, Inc. SLI Study No. 3472.4.

3. SLI, Springborn Laboratories. 2002. A range-finding 90-day oral (gavage) toxicity study in Fischer 344 rats with nickel sulfate hexahydrate. Spencerville, OH: Springborn Laboratories, Inc. SLI Study No. 3472.6.

The following document was received from the Food Safety Commission of Japan:

1. FSCJ (Food Safety Commission of Japan), 2012. Risk assessment report nickel (beverages). FS/683/2012. English translation of an excerpt from the original full report.

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APPENDICES

Appendix A. Standard or certified reference materials

Table A1: Standards or certified reference materials relevant to total nickel analysis in food and water (in mg/kg dry mass or µg/L)

Food or water type	Descriptor (supplier)[a]	Certified value[b]
Dogfish muscle	DORM-2 (NRCC)	19.4 ± 3.1
Dogfish liver	DOLT-4 (NRCC)	0.97 ± 0.11
Fish protein	DORM-3 (NRCC)	1.28 ± 0.24
Lobster hepatopancreas	TORT-2 (NRCC)	2.50 ± 0.19
Lobster hepatopancreas (non-defatted)	LUTS-1 (NRCC)	1.34 ± 0.23
Seaweed	IAEA 140/TM (IAEA)	3.79 ± 0.41
Fish muscle	IAEA 407 (IAEA)	0.60 ± 0.05
Tuna fish	IAEA 436 (IAEA)	0.069 ± 0.030
Whey powder	IAEA 155 (IAEA)	0.54 ± 0.10
Typical diet	SRM 1548a (NIST)	0.369 ± 0.023
Spinach leaves	SRM 1570a (NIST)	2.142 ± 0.058
Tomato leaves	SRM 1573a (NIST)	1.59 ± 0.07
Bovine liver	SRM 1577c (NIST)	0.445 ± 0.092
Oyster tissue	SRM 1566b (NIST)	1.04 ± 0.09
Mussel tissue	ERM-CE278k (IRMM)	0.69 ± 0.15
White cabbage	BCR-679 (IRMM)	27.0 ± 0.8
Mixed polish herbs	INCT-MPH-2 (INCT)	1.57 ± 0.16
Tea leaves	INCT-TL-1 (INCT)	6.12 ± 0.52
Rice	GBW 10010 (IGGE)	0.27 ± 0.02
Wheat	GBW 10011 (IGGE)	0.06 ± 0.02
Maize flour	GBW 10012 (IGGE)	0.097 ± 0.014
Soya bean	GBW 10013 (IGGE)	4.0 ± 0.3
Cabbage	GBW 10014 (IGGE)	0.93 ± 0.10
Spinach	GBW 10015 (IGGE)	0.92 ± 0.12
Tea	GBW 10016 (IGGE)	3.4 ± 0.3
Chicken	GBW 10018 (IGGE)	0.15 ± 0.03
Apple	GBW 10019 (IGGE)	0.14 ± 0.05
Crab paste	LGC 7160 (LGC)	0.23 ± 0.11
Rice flour unpolished	10-a (NIES)	0.19 ± 0.03
Rice flour unpolished	10-b (NIES)	0.39 ± 0.04
Cod fish tissue	7402-a (NMIJ)	0.38 ± 0.05
Seaweed	7405-a (NMIJ)	2.2 ± 0.1
White rice flour	7502-a (NMIJ)	0.390 ± 0.022
Tea leaf powder	7505-a (NMIJ)	5.5 ± 0.3
River water	SLRS-5 (NRCC)	0.476 ± 0.064
Hard drinking water	ERM-CA011b (IRMM)	19.27 ± 0.68
Soft drinking water	ERM-CA022a (IRMM)	20.5 ± 1.6
Simulated freshwater	SRM 1643e (NIST)	62.41 ± 0.69
Natural water	SRM 1640a (NIST)	25.32 ± 0.14
Spiked/fortified water	NWTM-15.2 (LGC)	17.6
Spiked/fortified water	NWTM-23.4 (LGC)	4.95
Spiked/fortified water	NWTM-24.3 (LGC)	5.12
Spiked/fortified water	NWTM-27.3 (LGC)	2.42
Spiked/fortified water	NWTMDA-61.2 (LGC)	57.5
Spiked/fortified water	NWTMDA-64.2 (LGC)	263
Spiked/fortified water	NWTMDA-51.4 (LGC)	65.6
Spiked/fortified water	NWTMDA-53.3 (LGC)	311
Spiked/fortified water	NWTM-DWS.2 (LGC)	82.5

Table continued overleaf.

Table A1: Standards or certified reference materials relevant to total nickel analysis in food and water (in mg/kg dry mass or µg/L) (continued)

Food or water type	Descriptor (supplier)[(a)]	Certified value[(b)]
Water	NIM-GBW08608 (LGC)	61
Simulated rain water	NWTRAIN-04 (LGC)	0.910
Surface water	SPS-SW1 (LGC)	10.0 ± 0.1
Surface water	SPS-SW2 (LGC)	50.0 ± 0.3
Water	NCS ZC76308 (LGC)	62 ± 2

a): IAEA: International Atomic Energy Agency (Austria); IGGE: Institute of Geophysical Exploration (China); INCT: Institute of Nuclear Chemistry and Technology (Poland); IRMM: Institute for Reference Materials and Measurements (Belgium); LGC: LGC (UK); NIST: National Institute of Standards and Technology (USA); NIES: National Institute for Environmental Studies (Japan); NMIJ: National Metrology Institute of Japan (Japan); NRCC: National Research Council of Canada (Canada).

(b): ± the uncertainty usually given as 95 % confidence interval.

Appendix B. Occurrence values used for chronic and acute exposure to nickel

Table B1: Occurrence values (µg/kg) used for chronic and acute exposure to nickel through food consumption. Values were rounded off to two significant figures.

Food commodities[a]	N	% LC[b]	Mean		95th percentile[d]		Groups[e]
			LB[c]	UB[c]	LB	UB	
Beer	170	82	1.0	7.2	7.1	21	Alcoholic beverages
Malt drink	17	94	470	1 100	-	-	
Beer and beer-like beverage (unspecified)	49	71	56	270	-	-	
Spirits and liqueurs	110	94	2.1	16	25	25	
Wine	301	55	19	55	62	210	
Wine-like drinks	240	64	36	54	150	150	
Alcoholic mixed drinks[f]			19	41	71	120	
Alcoholic beverages (unspecified)[f]			19	41	71	120	
Butter	61	56	78	92	290	290	Animal and vegetable fats and oils
Pork lard (Schmaltz)	65	58	330	330	360	360	
Cocoa butter	2	0	6 900	6 900	-	-	
Other vegetable fat than cocoa butter	5	60	360	560	-	-	
Margarine and similar products	78	58	350	490	770	770	
Vegetable oil	151	60	305	360	250	320	
Animal and vegetable fats and oils (unsp.)[f]			320	380	360	510	
Beans-based meals	2	0	1 400	1 400	-	-	Composite food (including frozen products)
Composite food (unspecified)	63	10	140	150	440	440	
Unspecified bottled water	1 130	79	1.3	2.6	7.0	7.0	Drinking water
Carbonated mineral water	2 363	65	7.0	8.0	16	16	
Still mineral water	751	80	0.9	2.2	5	5	
Tap water	1 888 0	94	0.1	1.1	1.0	1.0	
Water ice (for consumption)	11	73	0.2	0.4	0.8	0.8	
Well water	435	49	1.3	2.1	8.0	8.0	
Drinking water (unspecified)	2 130	84	2.1	2.6	5.2	5.2	
Eggs and egg products	115	74	38	57	180	180	Eggs and egg products
Fish meat	545	69	56	84	210	260	Fish and other seafood
Fish offal	17	6	99	99	-	-	
Crustaceans	69	39	43	180	130	580	
Water molluscs	51	2	390	390	-	-	
Fish products[f]			77	110	330	390	
Unspecified fish and other seafood	32	88	8.2	32	-	-	
Cereal-based food for infants and young children	69	41	190	290	640	640	Food for infants and small children
Follow-on formulae, powder	58	55	56	99	-	-	
Follow-on formulae, liquid[g]			7.0	13	-	-	
Infant formulae, powder	59		94	110	-	-	
Infant formulae, liquid[h]			12	13	-	-	
Fruit juice and herbal tea for infants and young children	6	17	30	36	-	-	
Ready-to-eat meal for infants and young children	45		36	91	-	-	
Yoghurt, cheese and milk-based dessert for infants and young children[i]			7.7	76	-	-	

Table continued overleaf.

Table B1: Occurrence values (µg/kg) used for chronic and acute exposure to nickel through food consumption. Values were rounded off to two significant figures. (continued)

Food commodities[a]	N	% LC[b]	Mean		95th percentile[d]		Groups[e]
			LB[c]	UB[c]	LB	UB	
Unspecified food for infants and small children	61	62	34	170	150	490	
Berries and small fruits	333	29	62	76	190	230	
Pome fruits	170	43	34	46	160	160	
Citrus fruits	78	32	52	59	150	150	
Dried fruits	13	23	140	160	-	-	
Apricots	17	0	540	540	-	-	
Peaches	29	3	92	99	-	-	
Plums	68	21	44	51	140	140	
Other stone fruits[j]			120	130	630	630	
Figs	3	0	1 800	1 800	-	-	Fruit and fruit products
Miscellaneous other than figs	195	30	51	68	158	190	
Jam, marmalade and other fruit spreads	14	93	2.4	650	-	-	
Other fruit products (excluding beverages)	35	23	83	130	-	-	
Fruit and fruit products (unspecified)[f]			68	91	210	300	
Fruit juice	340	35	27	45	120	120	
Fruit nectar	82	16	26	30	53	53	
Mixed fruit juice	10	10	34	37	-	-	Fruit and vegetable juices
Vegetable juice	63	14	46	55	89	99	
Concentrated fruit juice[k]			130	220	600	600	
Mixed fruit and vegetable juice[l]			35	58	99	120	
Bread and rolls	555	36	120	140	510	510	
Breakfast cereals	313	18	630	710	1 700	1 700	
Fine bakery wares	176	21	180	210	810	820	
Pasta (Raw)	150	27	120	160	410	540	
Buckwheat grain	133	2	1 200	1 200	2 400	2 400	
Millet grain	27	0	1 700	1 700	-	-	
Oats, grain	44	2	1 100	1 100	-	-	
Other grains	2 162	23	210	260	550	670	Grain and grain based products
Rye milling products	143	55	51	99	190	270	
Spelt milling products	23	26	1 600	1 600	-	-	
Wheat milling products (no bran)	360	44	71	110	260	260	
Wheat bran	41	5	640	650	-	-	
Oat milling products	41	0	1 100	1 100	-	-	
Corn milling products	54	26	120	150	-	-	
Other grain milling products	45	47	310	350	-	-	
Baking ingredients	11	45	130	190	-	-	
Flavourings or essences	22	45	170	220	-	-	
Herb and spice mixtures	19	0	1 800	1 800	-	-	
Herbs	122	7	490	490	2 800	2 800	Herbs, spices and condiments
Seasoning, extracts, condiments	79	75	120	190	790	1 100	
Spices	226	1	2 200	2 200	5 500	5 500	
Herbs, spices and condiments (unspecified)[f]			1 300	1 300	4 600	4 600	
Legumes, beans, green, with pods	12	0	450	450	-	-	
Legumes, beans, green, without pods	104	3	340	340	710	710	
Beans dried	51	0	2 900	2 900	-	-	Legumes, nuts and oilseeds
Lentils dried	64	0	2 100	2 100	3 600	3 600	
Peas dried	41	0	1 200	1 200	-	-	

Table continued overleaf.

Table B1: Occurrence values (µg/kg) used for chronic and acute exposure to nickel through food consumption. Values were rounded off to two significant figures. (continued)

Food commodities[a]	N	% LC[b]	Mean		95th percentile[d]		Groups[e]
			LB[c]	UB[c]	LB	UB	
Chick pea dried	11	0	620	620	-	-	
Soya beans dried	57	7	4 600	4 700	-	-	
Peanuts	148	5	3 500	3 600	11 000	11 000	
Other legumes, beans, dried[j]			3 100	3 100	11 000	11 000	
Almond, sweet	156	1	1 100	1 100	2 100	2 100	
Chestnuts	24	8	520	550	-	-	
Hazelnuts	48	0	2 200	2 200	-	-	
Other three nuts [j]			1 400	1 400	3 700	3 700	
Linseed	18	0	1 200	1 200	-	-	
Poppy seed	28	29	670	930	-	-	
Sesame seed	91	1	910	920	1 800	1 800	
Sunflower seed	68	1	2 500	2 500	6 100	6 100	
Rape seed	79	5	690	690	2 100	2 100	
Pumpkin seeds	121	1	1 800	1 800	2 600	2 600	
Other oilseeds[j]			1 500	1 600	3 400	3 400	
Livestock meat	629	61	96	120	330	330	Meat and meat products (including edible offal)
Poultry	231	76	63	99	130	210	
Game birds[m]			63	99	130	210	
Game mammals	264	64	170	190	580	580	
Preserved meat	8	50	18	26	-	-	
Sausages	277	59	150	240	170	610	
Meat specialities[n]			190	240	310	510	
Pastes, pâtés and terrines[n]			190	240	310	510	
Mixed meat[n]			190	240	310	510	
Edible offal, game animals	45	49	140	160	-	-	
Beef kidney	18	72	17	51	-	-	
Beef liver	303	57	120	140	410	410	
Giblets (chicken, turkey, duck, goose)	57	91	4.6	49	-	-	
Mutton/lamb liver	19	42	1 300	1 300	-	-	
Pork kidney	102	81	30	190	190	510	
Pork liver	187	83	970	1 100	190	510	
Other edible offal, farmed animals[j]			350	420	320	510	
Cheese	145	59	90	110	320	320	Milk and dairy products
Fermented milk products	58	85	7.7	76	-	-	
Liquid milk	355		21	31	91	91	
Dried milk[o]			230	350	990	990	
Evaporated milk[o]			61	94	270	270	
Condensed milk[o]			61	94	270	270	
Milk and milk product imitates	50	8	450	490	-	-	
Soft drinks	35	31	37	41	-	-	Non-alcoholic beverages (excepting milk based beverages)
Peppermint	47	11	560	560	-	-	
Rooibos leaves	42	24	180	190	-	-	
Other tea and herbs for infusions (solid)[j]	105		760	760	4 200	4 200	
Tea (Infusion)[p]			7.6	7.6	42	42	
Cocoa beans and cocoa products (solid)	238	0	9 500	9 500	12 000	12 000	
Cocoa beverage [q]			160	160	210	210	
Coffee beans and coffee products (solid)	83	2	1 200	1 200	3 100	3 100	
Coffee beverage[r]			68	68	170	170	

Table continued overleaf.

Table B1: Occurrence values (µg/kg) used for chronic and acute exposure to nickel through food consumption. Values were rounded off to two significant figures. (continued)

Food commodities[a]	N	% LC[b]	Mean		95th percentile[d]		Groups[e]
			LB[c]	UB[c]	LB	UB	
Coffee drink, espresso[s]			170	170	450	450	
Instant coffee, liquid[t]			21	21	52	52	
Food for sports people (labelled as such)	47	43	1 800	2 100	-	-	
Medical food	67	42	170	190	670	680	
Dietetic food for diabetics (labelled as such)	37	5	1 600	1 600	-	-	
Vitamin supplements	25	28	920	940	-	-	Products for special nutritional use
Mineral supplements	45	36	4 700	4 700	-	-	
Combination of vitamins and minerals supplements	116	22	3 200	3 200	16 000	16 000	
Plant extract formula	10	20	3 800	3 900	-	-	
Other dietary supplements	49	27	2 500	2 600	-	-	
Food for weight reduction	33	15	310	330	-	-	
Products for special nutritional use (unsp.)	42	5	290	290	-	-	
Ices and desserts	21	38	240	270	-	-	Snacks, desserts, and other foods
Other foods (foods which cannot be included in any other group)	30	87	82	820	-	-	
Snack food	22	50	31	57	-	-	
Main-crop potatoes	205	7	260	270	910	910	Starchy roots and tubers
New potatoes	22	18	49	55	-	-	
Mashed potatoes powder	87	53	55	160	210	250	
Other potatoes and potatoes products	279	29	44	71	120	250	
Other starchy roots and tubers	70	24	120	120	310	310	
Sugar and confectionery, unspecified	112	10	690	750	1 700	1 700	Sugar and confectionery
Sugars	95	88	11	150	66	1100	
Sugar substitutes	55	98	17	290	-	-	
Confectionery (non-chocolate)	226	40	310	530	980	1100	
Molasses and other syrups	8	38	290	550	-	-	
Honey	183	31	140	160	540	540	
Bitter-sweet chocolate	20	0	3 400	3 400	-	-	
Bitter chocolate[u]			3 400	3 400	-	-	
Chocolate, cream	9	0	1 300	1 300	-	-	
Pralines	35	0	1 300	1 300	-	-	
Filled chocolate[v]			1 300	1 300	-	-	
Chocolate coated confectionery[v]			1 300	1 300	-	-	
Milk chocolate	52	4	930	950	-	-	
Chocolate (Cocoa) products, except white chocolate	374	0	3 800	3 800	6 100	6 100	
Chocolate bar[v]			1 300	1 300	-	-	
Cooking chocolate[w]			3 800	3 800	6 100	6 100	
Dietetic chocolate[w]			3 800	3 800	6 100	6 100	
Chocolate sauce[w]			3 800	3 800	6 100	6 100	
Bulb vegetables	224	16	180	190	710	710	Vegetables and vegetable products (including fungi)
Fruiting vegetables	483	40	65	76	190	190	
Brassica vegetables	373	37	59	79	190	250	
Leaf vegetables	827	26	110	120	310	320	
Legume vegetables	9	0	320	320	-	-	
Stem vegetables (Fresh)	283	11	99	110	320	320	

Table continued overleaf.

Table B1: Occurrence values (µg/kg) used for chronic and acute exposure to nickel through food consumption. Values were rounded off to two significant figures. (continued)

Food commodities[a]	N	% LC[b]	Mean		95th percentile[d]		Groups[e]
			LB[c]	UB[c]	LB	UB	
Sugar plants	30	53	64	84	-	-	
Fungi, wild, edible	127	9	110	120	210	210	
Vegetable products	23	0	520	520	-	-	
Beetroot	55	24	82	89	-	-	
Radishes	60	43	23	31	76	76	
Carrots	303	19	160	170	760	760	
Celeriac	67	9	73	77	210	210	
Swedes	13	0	95	95	-	-	
Other root vegetables[j]			130	130	580	580	
Button mushroom	339	51	24	36	68	72	
Oyster mushroom	32	69	21	43	-	-	
Shiitake mushroom	19	26	120	130	-	-	
Other cultivated fungi	21	24	34	43	-	-	

(a): Within each food group and depending on their reported occurrence values, the samples were grouped at FoodEx level 1 (bold), level 2 (normal), level 3 (italics), before being linked with the EFSA Comprehensive Food Consumption Database.
(b): Percentage of left-censored data.
(c): LB= Lower bound, UB= Upper bound.
(d): The 95th percentile for samples with less than 60 observations is not shown as the results may not be statistically robust (EFSA, 2011b).
(e): Food samples were grouped at FoodEx level 1 to better explain their contribution to the dietary exposure to nickel;
(f): Mean value obtained from the average concentration of the food commodities grouped at FoodEx level 1.
(g): Occurrence values were calculated using a dilution factor of 8 applied on the samples of 'Follow-on formulae, powder'.
(h): Occurrence values were calculated using a dilution factor of 8 applied on the samples of 'Infant formulae, powder'.
(i): Since only one sample was reported, the occurrence value reported for 'Fermented milk' was used.
(j): Mean value obtained from the average concentration of the available food commodities grouped at FoodEx level 2.
(k): Occurrence values calculated multiplying by a factor of 5 the values reported for the samples of 'Fruit juice' at FoodEx level 2.
(l): Occurrence values calculated from the samples reported as 'Vegetable juice' and 'Fruit juice'.
(m): The occurrence values reported for 'Poultry' were used.
(n): The occurrence values reported for all samples of 'Meat and meat products (including edible offal)' at FoodEx level 1 were used.
(o): Occurrence values for 'Dried milk' were calculated multiplying the samples of 'Liquid milk' by a factor of 11, and by a factor of 3 to obtain the occurrence values of 'Evaporated milk' and 'Condensed milk'.
(p): Occurrence values were calculated using a dilution factor of 100 applied on the samples of 'Tea and herbs for infusions (solid)' at FoodEx level 2.
(q): Occurrence values were calculated using a dilution factor of 60 applied on the samples of 'Cocoa beans and cocoa products (solid)' at FoodEx level 2.
(r): Occurrence values were calculated using a dilution factor of 18 applied on the samples of 'Coffee beans and coffee products (solid)' at FoodEx level 2.
(s): Occurrence values were calculated using a dilution factor of 7 applied on the samples of 'Coffee beans and coffee products (solid)' at FoodEx level 2.
(t): Occurrence values were calculated using a dilution factor of 63 applied on the samples of 'Coffee beans and coffee products (solid)' at FoodEx level 2.
(u): The occurrence values reported for 'Bitter-sweet chocolate' were used.
(v): The occurrence values reported for 'Pralines' were used.
(w): The occurrence values reported for 'Chocolate (Cocoa) products, except white chocolate' were used.

Appendix C. Acute and chronic exposure assessment

Table C1: Dietary surveys considered for the chronic and acute dietary exposure assessment with the available number of subjects (for chronic exposure) and number of days (for acute exposure) in the different age classes

Code[a]	Country	Dietary survey[b]	Method	Days	Age	Number of subjects[c]/days[d]						
						Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
AT	Austria	ASNS	24-hour recall	1	19–65	-				-/2123		
BE/1	Belgium	Diet National 2004	24 h dietary recall	2	15–105				584/1 187	1 304/2 648	518/1 045	712/1 448
BE/2	Belgium	Regional Flanders	Food record	3	2–5		36[e]/108	625/1 875				
BG/1	Bulgaria	NUTRICHILD	24-hour recall	2	0.1–5	860/172	428/867	433/856				
BG/2	Bulgaria	NSFIN	24-hour recall	1	> 16				-/162	-/691	-/151	-/200
CY	Cyprus	Childhealth	Dietary record	3	11–18				303/909			
CZ	Czech Republic	SISP04	24-hour recall	2	4–64			389/798	298/596	1 666/3 332		
DE/1	Germany	DONALD 2006-2008	Dietary record	3	1–10		261/783	660/1 980				
DE/2	Germany	National Nutrition	24-hour recall	2	14–80				1 011/2 022	10 419/20 838	2 006/4 012	490/980
DK	Denmark	Danish Dietary Survey	Food record	7	4–75			490/3 426	479/3 398	2 822/19	309/2 159	20[e]/140
EL	Greece	Regional Crete	Dietary record	3	4–6			839/2 508				
ES/1	Spain	AESAN	Food record	3	18–60					410/828		
ES/2	Spain	AESAN-FIAB	24-hour recall	2	17–60				86/226	981/2 748		
ES/3	Spain	NUT INK05	24-hour recall	2	4–18			399/798	651/1 302			
ES/4	Spain	enKid	24-hour recall	2	1–14		17[e]/34	156/312	209/418			
EE	Estonia	NDS_1997	24-hour recall	1	19–64					-/1 866		
FI/1	Finland	DIPP	Food record	3	1–6		497/1 486	933/2 773				
FI/2	Finland	FINDIET 2007	48-hour recall	2	25–74					1 575/3 150	463/926	
FI/3	Finland	STRIP	Food record	4	7–8			250/1 000				
FR	France	INCA2	Food record	7	3–79			482/3 315	973/6 728	2 276/15 727	264/1 824	84/571
HU	Hungary	National Repr Surv	Food record	3	18–96					1 074/3 222	206/618	80/240
IE	Ireland	NSFC	Food record	7	18–64					958/6 706		
IT	Italy	INRAN-SCAI 2005–06	Food record	3	0.1–98	16[e]/48	36[c]/108	193/579	247/741	2 313/6 939	290/870	228/684
LV	Latvia	EFSA_TEST	24-hour recall	2	7–66			189/377	470/949	1 306/2 655		
NL/1	Netherlands	DNFCS 2003	24 h dietary recall	2	2–6					750/1 500		
NL/2	Netherlands	VCP kids	Food record	3	19–30		322/644	957/1 914				
PO	Poland	IZZ_FAO_2000	24-hour recall	1	1–96		-/79	-/409	-/666	-/2 527	-/329	-/124
SE/1	Sweden	RIKSMATEN 1997-98	Food record	7	18–74					1 210/8 466		
SE/2	Sweden	NFAn	24-hour recall	4	3–18			1 473/5 875	1 018/4 047			
SK	Slovakia	SK_MON_2008	24-hour recall	1	19–59					-/2 763		
SI	Slovenia	CRP_2008	24-hour recall	1	18–65					-/407		
UK	United Kingdom	NDNS	Food record	7	19–64					1 724/12 068		

(a): Codes to be used consistently in all tables on exposure assessment.
(b): More information on the dietary surveys is given in the Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).
(c): Number of available subjects for chronic exposure assessment in each age class.
(d): Number of available days for acute exposure assessment in each age class.
(e) 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b).

Table C2: Mean and 95th percentile (P95) chronic dietary exposure to nickel (µg/kg body weight (b.w.) per day) for total population in lower-bound (LB) and upper-bound (UB) scenario

Code[a]	Range of dietary exposure (LB – UB) (µg/kg b.w. per day)													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
BE/1							3.4-4.0	7.0-7.9	2.9-3.4	5.5-6.2	2.6-3.2	4.8-5.8	2.5-3.1	4.8-5.7
BE/2			9.6-11.9	-[b]	7.1-9.0	12.3-14.7								
BG	3.3-5.6	5.6-12.3	7.4-10.3	12.5-16.3	7.5-9.7	12.5-16.5								
CY							3.1-3.5	5.6-5.9						
CZ					8.0-9.5	16.0-17.8	4.9-5.9	10.7-12.3	2.6-3.1	5.1-5.9				
DE/1			5.3-7.3	8.7-10.8	5.9-7.3	10.3-12.7								
DE/2							2.7-3.4	5.8-7.0	2.6-3.4	5.2-6.9	2.3-3.0	4.3-5.6	2.2-3.0	4.0-5.4
DK					5.7-7.4	9.1-11.5	3.2-4.1	5.8-7.1	2.7-3.4	4.6-5.5	2.5-3.2	4.4-5.3	2.4-3.1	-[b]
EL					7.5-8.6	15.7-16.9								
ES/1									3.0-3.5	5.9-6.7				
ES/2							3.6-4.1	7.4-7.8	3.0-3.6	6.1-6.9				
ES/3					7.5-8.8	14.3-15.5	4.8-5.6	9.5-10.3						
ES/4			11.0-13.1	-[b]	8.1-9.5	16.5-18.2	4.8-5.6	9.9-11.2						
FI/1			7.1-9.3	14.7-20.1	6.4-8.4	12.1-15.2								
FI/2									2.4-3.0	4.5-5.4	2.0-2.6	3.6-4.4		
FI/3					6.7-8.2	10.9-13.0								
FR					8.2-9.9	15.5-18.2	4.0-4.9	8.0-9.3	2.8-3.4	5.1-6.1	2.6-3.2	4.7-5.5	2.4-3.1	4.3-5.1
HU									2.9-3.5	5.1-5.8	2.5-3.0	4.0-4.7	2.7-3.2	4.2-4.9
IE									2.2-2.8	3.7-4.7				
IT	4.1-6.3	-[b]	6.2-8.1	-[b]	6.0-7.2	13.1-14.6	3.5-4.1	7.4-8.1	2.4-2.9	4.6-5.3	2.2-2.7	3.8-4.6	2.3-2.8	4.4-5.0
LV					4.9-5.9	10.0-11.3	3.4-4.1	6.9-8.0	2.2-2.7	4.6-5.2				
NL/1									2.9-3.6	5.2-5.9				
NL/2			7.8-10.4	13.5-18.5	6.6-8.7	11.8-14.3								
SE/1									2.8-3.5	4.9-5.8				
SE/2					5.8-7.4	10.2-12.5	3.7-4.6	7.1-8.4						
UK									2.2-2.8	4.1-4.9				

(a): Details on the dietary surveys and the number of subjects are given in Table C1.
(b): 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust.

Table C3: Average and 95th percentile acute dietary exposure to nickel (upper bound estimates)

Age class	Survey	Number of consumption days[(a)]	Average acute exposure[(a)]	95th percentile exposure[(a)]
Infants	INRAN_SCAI_2005_06	48	6.4 (5.0-7.7)	-
	NUTRICHILD	1 720	5.6 (5.4-6.0)	15.1 (14.3-15.9)
Toddlers	Regional_Flanders	108	14.3 (13.2-15.5)	35.0 (26.8-47.2)
	NUTRICHILD	856	10.4 (10.0-10.9)	24.9 (22.2-27.8)
	DIPP_2003_2006	1 486	9.3 (9.0-9.6)	20.9 (19.8-22.0)
	DONALD_2006_2008	783	7.5 (7.0-8.1)	16.6 (15.0-18.2)
	INRAN_SCAI_2005_06	108	8.1 (7.1-9.7)	21.5 (16.1-27.7)
	VCP_kids	644	11.1 (10.5-11.8)	24.9 (22.5-28.5)
	IZZ_FAO_2000	79	12.1 (10.3-15.3)	28.8 (22.4-44.6)
	enKid	34	13.2 (11.5-17.0)	-
Other children	Regional_Flanders	1 875	10.2 (9.9-10.8)	22.9 (21.7-24.7)
	NUTRICHILD	867	9.8 (9.3-10.6)	23.7 (21.3-26.0)
	SISP04	778	9.4 (9.0-10.0)	22.5 (20.6-25.0)
	Danish_Dietary_Survey	3 426	7.7 (7.6-7.9)	16.6 (15.8-17.7)
	DIPP_2003_2006	2 773	8.4 (8.2-8.7)	18.7 (17.9-19.5)
	STRIP	1 000	8.2 (7.9-8.7)	18.8 (17.5-21.2)
	INCA2	3 315	10.4 (10.1-10.7)	25.5 (24.4-26.6)
	DONALD_2006_2008	1 980	8.0 (7.7-8.4)	18.0 (16.6-19.1)
	Regional_Crete	2 508	8.9 (8.6-9.2)	29.7 (27.8-31.8)
	INRAN_SCAI_2005_06	579	7.2 (6.8-7.6)	18.4 (16.5-20.4)
	EFSA_TEST	377	6.0 (5.5-6.8)	15.5 (13.5-18.2)
	VCP_kids	1 914	9.5 (9.2-9.8)	21.2 (19.6-22.3)
	IZZ_FAO_2000	409	10.8 (9.8-12.6)	23.7 (20.9-26.8)
	enKid	312	9.8 (9.0-12.5)	23.3 (20.3-27.0)
	NUT_INK05	798	9.2 (8.9-9.7)	20.6 (19.1-22.4)
	NFA	5 875	7.5 (7.3-7.6)	17.7 (17.1-18.5)
Adolescents	Diet_National_2004	1 187	4.5 (4.3-4.9)	11.3 (10.4-12.2)
	NSFIN	162	4.3 (3.8-5.6)	11.4 (9.0-14.6)
	Childhealth	909	3.6 (3.4-3.8)	11.0 (10-12.1)
	SISP04	596	5.9 (5.6-6.3)	15.1 (13.4-16.9)
	Danish_Dietary_Survey	3 348	4.3 (4.2-4.4)	10.1 (9.7-10.7)
	INCA2	6 728	5.2 (5.1-5.3)	13.8 (13.4-14.3)
	National_Nutrition_Survey_II	2 022	3.4 (3.1-3.7)	8.6 (8.0-9.1)
	INRAN_SCAI_2005_06	741	4.1 (3.9-4.4)	11.4 (9.5-13.0)
	EFSA_TEST	949	4.1 (3.9-4.6)	10.7 (10.0-12.1)
	IZZ_FAO_2000	666	7.2 (6.7-7.8)	16.1 (15.0-17.7)
	AESAN_FIAB	226	4.3 (4.0-4.8)	10.7 (9.3-12.7)
	enKid	418	5.8 (5.4-6.5)	14.0 (12.3-15.7)
	NUT_INK05	1 302	5.7 (5.5-5.8)	13.3 (12.3-14.4)
	NFA	4 047	4.6 (4.5-4.8)	11.7 (11.2-12.2)
Adults	ASNS	2 123	3.8 (3.5-4.0)	9.0 (8.3-9.5)
	Diet_National_2004	2 648	3.7 (3.5-3.9)	9.0 (8.4-9.5)
	NSFIN	691	3.0 (2.8-3.5)	8.5 (7.5-9.6)
	SISP04	3 332	4.0 (4.1-4.8)	11.8 (10.6-13.8)
	Danish_Dietary_Survey	19 722	3.4 (3.4-3.4)	7.6 (7.4-7.7)
	NDS_1997	1 866	3.0 (2.9-3.2)	7.9 (7.3-8.7)
	FINDIET_2007	3 150	3.0 (2.9-3.1)	6.6 (6.3-7.0)
	INCA2	15 727	3.5 (3.4-3.6)	8.9 (8.7-9.1)
	National_Nutrition_Survey_II	20 838	3.4 (3.3-3.5)	7.8 (7.6-8.0)
	National_Repr_Surv	3 222	3.5 (3.3-3.7)	8.4 (7.9-8.9)
	NSIFCS	6 706	2.8 (2.8-2.9)	6.4 (6.2-6.6)
	INRAN_SCAI_2005_06	6 939	3.1 (3.0-3.2)	7.9 (7.6-8.4)

Table C3: Average and 95th percentile acute dietary exposure to nickel (upper bound estimates) (continued)

Age class	Survey	Number of consumption days[(a)]	Average acute exposure[(a)]	95th percentile exposure[(a)]
Elderly	EFSA_TEST	2 655	2.7 (2.6-2.9)	6.8 (6.5-7.1)
	DNFCS_2003	1 500	3.9 (3.7-4.2)	9.1 (8.4-9.8)
	IZZ_FAO_2000	2 527	5.1 (4.8-5.7)	11.6 (11.0-12.3)
	SK_MON_2008	2 763	2.8 (2.7-3.1)	7.1 (6.7-7.6)
	CRP_2008	407	3.1 (2.7-3.6)	8.4 (6.9-10.4)
	AESAN_FIAB	2 748	3.6 (3.5-3.7)	9.3 (8.7-9.7)
	AESAN	828	3.5 (3.3-3.8)	9.0 (8.0-9.9)
	Riksmaten_1997_98	8 466	3.7 (3.6-3.8)	9.0 (8.8-9.3)
	NDNS	12 068	2.8 (2.7-2.8)	6.8 (6.6-7.0)
	Diet_National_2004	1 045	3.3 (3.1-3.6)	7.6 (6.9-8.1)
	NSFIN	151	2.5 (2.2-2.9)	6.5 (4.9-9.2)
	Danish_Dietary_Survey	2 159	3.2 (3.2-3.4)	6.9 (6.6-7.3)
	FINDIET_2007	926	2.6 (2.5-2.8)	5.5 (5.1-6.0)
	INCA2	1 824	3.3 (3.2-3.4)	8.2 (7.6-8.9)
	National_Nutrition_Survey_II	4 012	3.1 (2.9-3.2)	6.7 (6.4-7.0)
	National_Repr_Surv	618	3.0 (2.8-3.3)	7.3 (6.4-8.3)
	INRAN_SCAI_2005_06	870	2.7 (2.6-2.9)	6.5 (5.7-7.5)
	IZZ_FAO_2000	329	4.0 (3.5-5.2)	9.1 (7.9-10.7)
Very elderly	Diet_National_2004	1 448	3.2 (3.0-3.5)	7.4 (6.9-7.9)
	NSFIN	200	2.7 (2.4-3.1)	7.1 (5.5-9.5)
	Danish_Dietary_Survey	140	3.1 (2.9-3.4)	6.6 (5.5-8.5)
	INCA2	571	3.1 (2.9-3.4)	8.0 (6.7-9.5)
	National_Nutrition_Survey_II	980	3.0 (2.8-3.2)	6.5 (5.9-7.3)
	National_Repr_Surv	240	3.2 (2.9-3.8)	7.5 (6.1-9.5)
	INRAN_SCAI_2005_06	684	2.8 (2.6-3.0)	7.2 (6.2-8.4)
	IZZ_FAO_2000	124	4.0 (3.2-6.6)	8.7 (6.6-11.3)

(a): in brackets the 95 % confidence interval.
(b): number of reported consumption days in the EFSA Comprehensive Database.
(c): number of subjects participating in the dietary survey.

Table C4: Range of average contribution of different foods (grouped at FoodEx Level 1) to acute dietary exposure to nickel in the European population

Food groups	Range of contribution (%)[a]							Number of times with the highest contribution by age class[b]					
	Infants [c] (n = 2)	Toddlers (n = 8)	Other children (n = 16)	Adolescents (n = 14)	Adults (n = 21)	Elderly (n = 9)	Very elderly (n = 8)	I	T	OC	A	E	VE
Alcoholic beverages	0.0	0.0	0.0 [0.0–0.3]	0.1 [0.0–0.9]	1.6 [0.5–7.6]	2.8 [0.4–4.1]	2.6 [0.1–3.9]						
Animal and vegetable fats and oils	0.9–1.5	2.4 [1.3–4.6]	2.5 [1.2–4.8]	2.7 [1.3–5.3]	3.6 [1.5–5.6]	4.3 [3.0–5.9]	4.2 [3.2–6.2]						
Composite food (including frozen products)	0.1–3.2	1.8 [0.3–8.8]	3.1 [0.0–25.4]	3.6 [0–18.6]	1.2 [0–22.3]	0.7 [0.1–8.6]	0.7 [0.0–10.1]			1	1		
Drinking water	2.2–4.8	0.6 [0.2–1.2]	0.4 [0.0–0.8]	0.4 [0.0–1.9]	0.6 [0.2–1.7]	0.7 [0.0–1.3]	0.5 [0.0–1.2]						
Eggs and egg products	0.0–0.3	0.5 [0.2–0.7]	0.4 [0.0–0.8]	0.4 [0.0–0.9]	0.5 [0.1–1.0]	0.6 [0.2–1.2]	0.7 [0.2–0.8]						
Fish and other seafood	0.0–0.3	0.4 [0.0–2.7]	0.6 [0.2–3.7]	0.9 [0.2–4]	0.9 [0.3–4.8]	1.0 [0.2–3.1]	1.1 [0.2–1.9]						
Food for infants and small children	34.5	7.5 [1.8–19.6]	0.3 [0.1–1.8]	0.0 [0.0–0.1]	0.0 [0.0–0.8]	0.0	0.0	1					
Fruit and fruit products	3.9–4.6	8.0 [3.8–10.8]	6.3 [3.7–9.1]	5.5 [3.5–8.2]	6.8 [3.9–9.4]	8.9 [5.9–14.2]	10.1 [5.9–15.6]						
Fruit and vegetable juices	1.4–5.8	3.4 [1.1–9.0]	3.4 [1.5–9.0]	1.9 [1.2–7.1]	1.4 [0.5–4.1]	0.8 [0.2–2.8]	0.7 [0.2–2.8]						
Grain and grain based products	3.8–7.9	20.9 [14.4–28.8]	21 [17.1–29.4]	24.9 [20.1–28.6]	21.2 [13.6–29.4]	19.8 [16.7–26.6]	21.3 [18.3–26.6]		5	11	17	7	7
Herbs, spices and condiments	0.2–0.9	0.5 [0.0–1.9]	0.7 [0.1–2.0]	0.8 [0.1–3.4]	1.1 [0.3–4.6]	1.4 [0.2–3.1]	1.1 [0.2–3.1]						
Legumes, nuts and oilseeds	1.5	2.7 [0.7–6.7]	2.3 [0.7–9.2]	4.1 [1.0–11.7]	4.3 [1.5–10.9]	4.4 [1.8–9.2]	3.1 [1.5–10.1]						
Meat and meat products (including edible offal)	0.6–1.7	4.1 [2.8–7.4]	5.2 [2.6–6.7]	6.3 [4.4–8.3]	6.4 [4.3–9.7]	5.4 [4.7–9.3]	5.0 [3.6–9.2]						
Milk and dairy products	21.0–41.9	17.8 [13.6–19.9]	12.4 [6.5–19.6]	7.6 [4.6–13.9]	7.5 [3.5–12.3]	8.0 [5.7–12.8]	7.7 [5.5–14.6]	1					
Non-alcoholic beverages	0.2–0.5	2.1 [0.6–26.6]	8.8 [2.1–27.4]	10.1 [4.0–25.8]	11.1 [6.2–21]	10.1 [2.6–24.8]	9.7 [1.9–22.6]		1	2	2	1	1
Products for special nutritional use	1.0	0.1 [0.0–0.5]	0.0 [0.0–0.5]	0.2 [0.0–1.5]	0.4 [0.0–1.8]	0.3 [0.1–2.4]	0.8 [0.1–1.9]						
Snacks, desserts, and other foods	0.8	2.6 [0.4–4.3]	3.1 [1.9–4.7]	2.0 [1.2–3.9]	1.3 [0.1–2.9]	0.8 [0.1–1.6]	0.9 [0.3–2.2]						
Starchy roots and tubers	1.0–4.5	3.4 [1.8–13.8]	3.2 [1.7–17.2]	3.9 [1.8–19.1]	4.2 [1.9–19.3]	5.0 [2.5–20.5]	5.6 [2.6–19.4]				1	1	
Sugar and confectionery	1.8–6.4	5.1 [2.6–24.2]	10.8 [5.4–24.3]	8.4 [5.5–20.3]	6.8 [3.0–15.1]	4.0 [1.5–7.6]	3.9 [2.7–6.7]		2	2			
Vegetables and vegetable products	3.3–4.3	6.9 [4.4–10.8]	5.4 [2.6–11.1]	6.9 [1.9–11.9]	9.6 [3.0–15.0]	11.8 [6.8–16.3]	12.5 [6.3–15.5]						

(a): Median and range of average contribution of different food groups to acute exposure to nickel in the population.
(b): I: infants; T: toddlers; OC: other children; A: adults; E: elderly; VE: very elderly.
(c): As only two surveys were available the median was not calculated.

Appendix D. Acute toxicity studies with nickel compounds

Table D1: Oral LD_{50} values of nickel compounds

Substance	Species	LD_{50} mg substance/kg b.w.	LD_{50} mg Ni/kg b.w.	Reference
Ni chloride[(a)]	Rat	105	48	Mastromatteo (1986)
Ni chloride[(a)]	Rat	M: 430, F: 529	M: 105, F: 130	Itskova et al. (1969)
Ni choride hexahydrate[(a)]	Rat (SD)	M: 210, F: 175	M: 51, F: 43	FDRL (1983a)
Ni chloride hexahydrate[(a)]	Rat (SD)	500	125	Henderson et al. (2012)
Ni sulphate[(a)]	Rat		M: 46, F: 39	Mastromatteo (1986)
Ni sulphate[(a)]	Rat	500	190	Kosova (1979)
Ni sulphate[(a)]	Rat	M: 325, F: 275	M: 73, F: 61	FDRL (1983b)
Ni sulphate hexahydrate[(a)]	Rat	300	67	Mastromatteo (1986)
Ni sulphate hexahydrate[(a)]	Rat (SD)	362	81	Henderson et al. (2012)
Ni nitrate[(a)]	Rat	1 620	330	Smyth et al. (1969)[(f)]
Ni nitrate hexahydrate[(a)]	Rat (SD)	> 200	> 40	ECHA (2003)
Ni acetate[(b)]	Rat	355	118	ATSDR (1985)
Ni acetate[(b)]	Rat	M: 350, F: 360	M: 119, F: 116	Haro et al. (1968)
Ni acetate tetrahydrate[(b)]	Rat	550	325	Henderson et al. (2012)
Ni carbonate[(c)]	Rat	M: 1 305, F: 840	M: 625, F: 402	FDRL (1983c)
Ni fluoride tetrahydrate	Rat	310	109	Henderson et al. (2012)
Ni sulfamate tetrahydrate	Rat	1 098	198	Henderson et al. (2012)
Ni hydroxide[(d)]	Rat	M: 1 500, F: 1 700	M: 915, F: 1 037	FDRL (1983d)
Ni hydroxide[(d)]	Rat	1 600	1 021	Mastromatteo (1986)
Ni oxide[(e)]	Rat	> 5 000	> 3 930	Mastromatteo (1986)
Ni oxide[(e)]	Rat	> 5 000	> 3 930	FDRL (1983f)
Ni oxide black or green[(e)]	Rat (SD)	8 796 –>11 000	6 910–> 8 650	Henderson et al. (2012)
Ni dihydroxide[(e)]	Rat (SD)	5 000	3 150	Henderson et al. (2012)
Ni trioxide[(e)]	Rat	> 5 000	> 3 548	FDRL (1983e)
Ni hydroxycarbonate[(e)]	Rat	2 000	1 140	Henderson et al. (2012)
Ni sulphide[(e)]	Rat	> 5 000	> 3 233	Mastromatteo (1986)
Ni subsulphide[(e)]	Rat	> 5 000	> 3 665	Mastromatteo (1986)
Ni subsulphide (crystalline)[(e)]	Rat	> 5 000	> 3 663	FDRL (1983g)
Ni subsulphide (amorphous)[(e)]	Rat	> 5 000	> 3 620	FDRL (1983h)
Ni subsulphide[(e)]	Rat	> 11 000	> 8 060	Henderson et al. (2012)

b.w.: body weight; F: female; M: male; SD: Sprague-Dawley.
(a): very soluble.
(b): soluble.
(c): slightly soluble
(d): very slightly soluble.
(e): insoluble.
(f): study carried out in non-fasted animals, probably an underestimate of the acute toxicity.

Appendix E. Repeated toxicity studies with nickel compounds

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
4-week Oral (diet) Young mice (Webster swiss) 6 M + 6 F/dose Ni acetate 0, 1 100 and 1 600 mg/kg food = 0, 200 and 320 mg Ni/kg b.w. per day	-	200 mg Ni/kg b.w. per day	Decrease b.w. at HD in M and both doses in F. Decrease feed consumption in M. Effects were observed on kidney and liver enzyme activities.	Klimisch score[(a)]: 3 Limited study: determination of the influence of toxic levels of Ni on feed utilization, growth and the activity of several enzymes. Only 2 tested doses.	Weber and Reid (1969)
28-day oral (drinking water) M Rat (Wistar) 10 M/dose $NiCl_2$ 0, 2.5, 5.0 and 10.0 mg/L = 0, 0.35, 0.7 and 1.4 mg Ni/kg b.w. per day	-	0.35 mg Ni/kg b.w. per day	Dose-rel. decrease b.w. gain. Decrease water consumption at HD. Decrease liver and kidney weights at HD. Dose-dependent hyperglycaemia. Decrease urea in serum at LD and MD and increase urea in urine at LD and HD. Increase leukocyte count at MD.	Klimisch score: 3 Limited information reported: b.w., water consumption, haematology, clinical chemistry, urinalysis. No data on gross pathology and histopathology Only M.	Weischer et al. (1980)
40-day oral (gavage) M mouse (Balb/c) 5 M/dose Ni chloride ($NiCl_2$) 0, 2, 8.2 and 16 mg/kg b.w. Equivalent to 0, 0.5, 2.0, 4.0 mg Ni/kg b.w. per day	-	0.5 mg Ni/kg b.w. per day	Dose-dependent decrease feed and water consumption. Decrease b.w. and liver weight at MD and HD. Liver: dose-related hepatocyte degeneration, nuclear pycnosis, cellular swelling and congestion of blood vessels (MD and HD), hypertrophy of hepatic cells (MD and HD), increases in apoptosis (HD) and severity of necrosis (HD). Increases in binucleated cells at all doses.	Klimisch: 2 Limited study: toxic effects of Ni on liver structure, only M.	Gathwan et al. (2013)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
6-week oral (diet) Rat (weanling O.S.U. brown) 6 M/dose Ni acetate 0, 100, 500 and 1 000 mg/kg diet (basal diet content: 0.21 mg/kg Ni) 0, 12, 60 and 120 mg Ni/kg b.w. per day	-	12 mg Ni /kg b.w. per day	Decreased b.w. gain at 2 HD, haemoglobin concentrations (mainly at HD) and packed cell volumes. Decreased cytochrome oxidase activity in heart not liver. Decreased AP activities in plasma, liver and heart at 2 HD. Dose-related increase Ni in plasma and RBC, heart, kidney (highest accumulation), liver and testes. Accumulation of Ni in blood components had no influence on the concentration of copper, but increased the iron and zinc concentrations.	Klimisch: 3 Limited study: b.w., haematology, clinical biochemistry (limited), only M. Effects of dietary Ni on enzyme activities and mineral content.	Whanger (1973)
8-week Oral (drinking water) Rat (Sprague Dawley) 10/dose $NiSO_4 \cdot 6H_2O$ 0 and 800 mg/L 0 and 72 mg Ni/kg b.w. per day	-	72 mg Ni/kg b.w. per day	Slight decrease b.w., decrease hepatic protein content, increase hepatic AP and ALT activities. Significant increase Ni, phosphorus and sulphur in liver tissue. Decrease Zn and Cu, Se and K in liver tissue, increase Fe.	Klimisch: 3 Limited study: liver toxicity in protein deficient rat. Only 1 dose.	Sidhu et al. (2005)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
13-week oral (drinking water) Male Rat (Sprague Dawley) 8 M/dose Nickel sulphate ($NiSO_4 \cdot 6H_2O$) 0, 0.02, 0.05, 0.1% = 0, 44.7, 111.75, 223.5 mg Ni/L = 0, 4, 10 and 20 mg Ni/kg b.w. per day	4 mg Ni/kg b.w. per day	10 mg Ni/kg b.w. per day	Slight decrease b.w. at HD. Decrease absolute and relative liver weights at 2 HD. Decrease absolute weight of testes and heart in treated animals. Increase absolute weight of kidneys, brain and spleen at HD. Increase relative spleen weights in all treated groups, relative kidney weights at LD and HD, and relative brain weight at HD. Increase absolute lung weights at LD and HD and increase relative lung weights at HD. Decrease total plasma proteins at 2 HD, and plasma albumin and globulins at HD. Decrease plasma glutamic pyruvic transaminase activity at HD. Effects on splenic lymphocytes T-cell subpopulations (CD4+, CD8+, ratio) and B cells and on thymocytes subpopulations T-cell subpopulations (CD4+, CD8+, ratio) and B cells at all doses. Decreases in urine volume and urine glucose at 2 HD, increased BUN at HD. Decrease AP activity in lung tissue at HD. Decrease AP activity in bronchoalveolar lavage fluid in treated animals, increase proteins in BALF at 2 HD. No damages to the testes as verified by measurements of the activities of testicular enzymes (AP, acid phosphatase, lactate dehydrogenase). No gross or microscopic changes in any tissues examined. Dose-dependant increase of Ni in different organs, not significant in most situations (kidneys > testes > lung ≈ brain > spleen > heart = liver).	Klimisch: 2 Limitations in the study: only M, only 8 animals/group, histopathological evaluation not performed on all tissues, absence of electron microscopy.	Obone et al. (1999)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
90-day Oral (drinking water) M Rat (Wistar) 7 M/dose $NiCl_2.6H_2O$ O, 300, 1 200 mg/L	300 mg $NiCl_2 \cdot 6H_2O$/L	1 200 mg $NiCl_2 \cdot 6H_2O$ /L	Decrease b.w. at HD. Increase lung weight at HD. Increase iron in liver, lungs and serum at LD and in liver, kidney and serum at HD.	Klimisch: 3 Limited information reported: b.w., water consumption, haematology, organ weights, Ni concentration in tissues. Only M.	Cempel (2004)
91-day Oral (gavage) Rat (Sprague Dawley) 30 M + 30 F/dose Ni chloride hexahydrate 0, 5, 35 and 100 mg Ni/kg b.w. per day	-	5 mg Ni/kg b.w. per day	Clinical signs of toxicity at HD. Mortality: 0, 2, 14 (6 M and 8 F) and 60/60 animals. Mortality at HD attributed to treatment and mortality of 3/6 M and 5/8 F at MD due to gavage errors. Lower b.w. at two HD in M and F and lower food consumption at two HD in M. Clinical signs of toxicity at HD: lethargy, ataxia, prostration, irregular breathing, blue coloration, discoloured extremities, cool body temperature, salivation, squinting and loose stools. Decreased incidence and occurrence of these toxic signs at MD. Significant increase WBC at LD and MD (not measured at HD) at interim sacrifice. Dose-related increase in platelet count in F, differences in differential leucocytes count (increases in neutrophils and decrease in lymphocytes) at MD in F. Dose-related decrease in glucose (significant at MD). Decrease absolute kidney, liver, spleen, brain (also relative) and heart weights in M at MD and decrease right kidney weight in F at MD. Increase relative testis weight at MD. Dose-related gastrointestinal tract abnormalities (discoloured contents, distension, stomach discoloration, ulceration and smooth mucosa) and lung abnormalities in treated animals. Macroscopic ulcerative gastritis and enteritis at HD. Pneumonitis in 6/19 M and 9/17 F in MD.	Klimisch: 2 GLP study Observations: mortality, b.w., food consumption, clinical signs of toxicity, haematology, ophthalmology, gross pathology organ weights, histopathology. Limitations: HD > MTD (all animals died)	American Biogenics Corporation (1988)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
90-day Oral (gavage) Rat (F344) 10 M + 10 F $NiSO_4$ hexahydrate 0, 50, 75, 100, 125 and 150 mg/kg b.w. per day, two HD M groups reduced to 30 and 15 mg/kg b.w. per day on d28 = 0, 11, 17, 22, 28(-7), 33-(3) mg Ni/kg b.w. per day	7 mg Ni/ kg b.w. per day	11 mg Ni/kg b.w. per day	Dose-related decrease b.w. gain, significant in M within the first 4 weeks at two HD. Exposures of M in these two groups were subsequently reduced to 30 and 15 mg/kg b.w. per day to ensure survival of animals. 1 HD F died on day 44. Clinical symptoms: post-dosing salivation, decreased activity (most pronounced within the first 2 weeks in HD groups) Only significant adverse effect: b.w. loss at ≥ 50 mg/kg b.w. per day (8–13 % lower compared to C). Variety of decreased absolute or increased relative organ weights. These effects were not accompanied by histopathological changes.	Klimisch: 2 GLP Study. Observations: b.w., clinical signs of toxicity, gross pathology, organ weights, histopathology	Rush (2002) SLI (2002)
25-week Oral (drinking water) M Rat (F344) 15 males Ni chloride hexahydrate 0, 600 mg/L = 0, 10.2 mg Ni/kg b.w. per day	10.2	-	No significant reduction b.w. No significant decrease in survival or effect on kidney weight	Klimisch: 3 Limited study: limited number of endpoints examined, Only M. Study of promoting effect of metal compounds on rat renal tumourigenesis (prior exposure to 500 ppm EHEN) Dose not known since exposed through drinking water and intake was not measured. Unclear if MTD was reached.	Kurokawa et al. (1985)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
180-day oral (drinking water) Female Mouse (B6C3F1) 10 F/dose Nickel sulphate (hexahydrate) 0, 1, 5 or 10 g/L = 0, 116, 286, and 396 mg Ni sulphate/kg b.w. per day 0, 33, 167 and 334 mg Ni/kg b.w. per day	-	33 mg Ni/kg b.w. per day	Decrease b.w. at HD (26 %) Decrease absolute liver weight in dosed animals Dose-rel. reduction absolute and relative thymus weight, even at the lowest dose. Kidney = major organ of Ni accumulation. Treatment-related increases in nephrosis at 2 HD (minimal to mild). Primary toxic effects expressed in the myeloid system: dose-related decreases in bone marrow cellularity, and in granulocyte macrophage and pluripotent stem-cells proliferative responses. Spleen: decreased extramedullary hematopoiesis and reduction number of splenic follicles; dose-rel. reduction in lymphoproliferative responses to the B-cell mitogen LPS.	Klimisch: 2 Evaluation of tissue disposition, myelopoietic and immunologic responses. Only F.	Dieter et al. (1988)
3- and 6-month oral (drinking water) Rat (Wistar) 10 M + 10 F/group 0, 100 mg Ni/L as nickel sulphate M: 0, 6.9 mg Ni/kg b.w. per day F: 0, 7.6 mg Ni/kg b.w. per day	-	6.9 mg Ni/kg b.w. per day	M: significant increase kidney weight after 6 months. F: increase albumin excretion after 6 months. No effect on the markers of tubular function. No effect on b.w. gain.	Klimisch: 3 Study of chronic nephrotoxicity. Limitations: considerable variability in response in M and F. Only 1 dose.	Vyskocil et al. (1994)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
2-year study Oral (diet) Rat (Wistar) 25 M + 25 F/dose Ni sulphate hexahydrate ($NiSO_4 \cdot 6H_2O$ containing 22.3 % Ni) 0, 100, 1000 and 2 500 mg Ni/kg food = 0, 5, 50 and 125 mg Ni/kg b.w. per day	5 mg Ni/kg b.w. per day	50 mg Ni/kg b.w. per day	Low survival rate in 2-year study (particularly in control and M HD). Decreased b.w. at two HD and sporadically at LD (partly resulting from lower food consumption). Increased relative heart weight and decreased rel. liver weight in F at two HD. No important storage of Ni in tissues.	Klimisch: 2 Limited number of necropsies due to high mortality. Observations: b.w., food consumption, haematology, gross examination, heart, spleen, kidney, liver and testes weights, histopathology: previous organs and lung, urinary bladder, stomach, small and large intestine, skeletal muscle, brain, skin, bone marrow, pituitary, thyroid, adrenal, pancreas and gonad.	Ambrose et al. (1976)
2-year study Oral (diet) Dog (Beagle) 3M + 3F Ni sulphate hexahydrate ($NiSO_4 \cdot 6H_2O$ containing 22.3 % Ni) 0, 100, 1000 and 2 500 mg Ni/kg food = 0, 1.8, 18 and 45 mg Ni/kg b.w. per day	18 mg Ni/kg b.w. per day	45 mg Ni/kg b.w. per day	Vomiting at HD during first 3 days. Decreased b.w. at HD. Slight decrease hematocrit and haemoglobin at HD (simple hypochromic anaemia). Marked polyuria in 2 dogs at HD. Increased rel. kidney and liver weight at HD. Lung lesions (multiple subpleural peripheral cholesterol granulomas, bronchiolectasis, emphysema, focal cholesterol pneumonia) at HD. Granulocytic hyperplasia of the bone marrow in 2 dogs at HD. No important storage of Ni in tissues.	Klimisch: 2 Observations: b.w., food consumption, haematology, gross pathology, heart, spleen, kidneys, liver and testes weights, histopathology: previous organs and lung, urinary bladder, stomach, small and large intestine, skeletal muscle, brain, skin, bone marrow, pituitary, thyroid, adrenal, pancreas and gonad.	Ambrose et al. (1976)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
104-week Oral (gavage) Rat (F344) 60 M + 60 F/dose $NiSO_4$ hexahydrate 0, 10, 30 and 50 mg/kg b.w. per day = 0, 2.2, 6.7 and 11.2 mg Ni/kg b.w. per day	2.2 mg Ni/kg b.w. per day	6.7 mg Ni/kg b.w. per day	Mortality: M: 60, 48, 50 and 57 %, F: 23, 33, 43 and 45 %, respectively. Higher rate of mortality in treated animals during first 24 weeks of the study (secondary to aspiration of Ni sulphate solution). Dose-related increase mortality in F. Dose-related decrease b.w., significant at two HD. No treatment-related effect on haematology, biochemistry, urinalysis parameters, gross pathology or histopathology. No carcinogenic effect observed	Klimisch: 2 GLP, OECD 451 Observations: mortality, clinical observations, b.w., food consumption, hematology, gross necropsy and histopathology. Limitation: high mortality not related to treatment during first 24 weeks of exposure. For both males and females, survival throughout the study was with a minimum of 78 % at 18 months and a minimum of 40 % survival by the study termination at 105 weeks. More than 25 animals survived by study termination (except in control males, where 24 survived)	Heim et al. (2007)
Life-time oral (drinking water) Mouse (Swiss Charles River CD) Ni acetate 0, 5 mg/L			Longevity was increased. No significant reduction of b.w. No tumorigenic effect observed.	Klimisch: 3 Very limited and poorly reported study. Only 1 dose;	Schroeder and Mitchener (1975)
Oral (drinking water) Rat (Long Evans) Ni acetate 0, 5 mg/L = 0.44 and 5.44 mg Ni/kg b.w. per day			No significant reduction in survival or b.w.	Klimisch: 3 Limited study. Only 1 dose.	Schroeder et al. (1974)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
36 month or until death oral (drinking water) Mouse (Swiss) Ni acetate 0 or 5 mg/L = 0, 0.45-0.51 mg Ni/kg b.w. per day			No significant reduction in survival or b.w.	Klimisch: 3 Limited study. Only 1 dose.	Schroeder et al. (1964)

AP: alkaline phosphatase; ALT: alanine transferase; BUN: blood urea nitrogen; BALF: bronchoalveolar lavage fluid; b.w. body weight; EHEN: N-ethyl-N-hydroxyethylnitrosamine; F: female; GLP: Good Laboratory Practice; HD: high dose; LD: low dose; LOAEL: lowest-observed-adverse-effect level; LPS: lipopolysaccharide; M: male; MD: mid dose; MTD: maximum tolerated dose; Ni: nickel; RBC: red blood cells; NOAEL: no-observed-adverse-effect level; WBC: white blood cells.

(a): Klimisch score: 1 = RWoR: reliable without restriction, 2 = RWR: reliable with restriction, 3 = NR: non reliable, 4 = NA: non assignable. Dose conversion calculated by the CONTAM Panel.

Appendix F. Developmental and reproductive toxicity studies with nickel compounds

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
Reproductive toxicity					
2-GEN study Oral (drinking water) Rat (CD) 0, 50, 250 or 500 mg Ni chloride hexahydrate/L 0, 6.0/6.2, 25/23 and 42/42 mg Ni/kg b.w. per day Add. dose of 1 000 mg Ni chloride hexahydrate/L eliminated after 2 weeks due to excessive toxicity Average exposure premating/mating period: Males 0, 4, 19 and 31 mg Ni/kg b.w. per day Females 0, 3, 12 and 22 mg Ni/kg b.w. per day Exposure ranges gestation period: 5-6, 22-26, 33-44 mg Ni/kg b.w. per day (average: 0, 6, 25 and 42 mg Ni/kg b.w. per day) Exposure ranges post natal period (GD20–PND 21) 4-13, 12-58, 14-98 mg Ni/kg b.w. per day Breeding: P0: 31-32 animals/sex/dose F1b: 30 M + 30 F/ group (0, 50 and 250 ppm), 22M + 19F at 500 ppm F1b: 15-19 litters/group (culling PND 21: 10 live pups/litter)	Parental toxicity: 25 Reproduction toxicity: 42 Offspring toxicity: 6	Parental toxicity: 42 Reproduction toxicity: - Offspring toxicity: 25	<u>Parents</u>: Stat. signif. decrease b.w. and liver weights (P0) in F at HD. Signif. reduction of relative food intake during first week of exposure to HD (P0) and during late pregnancy and lactation at 2 HD. Signif. reduction of water intake in M and F at certain time points at MD and HD (P0 and F1). P0 F: 1 death related to acute Ni toxicity at HD. F1: increase death between weaning and PND 42 in M at two HD and at HD in F. Signif. decrease b.w. in M and F at HD, decrease liver weight and increase lung weight in F at HD. Increase pituitary weight in M at two HD. No effect on gross pathology or histopathology, with the exception of that histiocytic infiltration of the lung tended to increase with dose for both sexes. No effect on reproductive performances, reproductive organ weights or histopathology of reproductive organs P0 F and F1 F: death and moribund sacrifices associated with complications of pregnancy occurred with an increased incidence at two HD. At two HD: increase gestation length. <u>Offsprings</u>: At HD: decreased live pups/litter in F1a, F1b and F2a (naturally-delivered litters), increase pup mortality, decrease pup b.w. At LD and MD, increase pup mortality and decrease live litter size in F1b (questionable). In F2b litter: no reduction in litter size observed (foetuses delivered by caesarean section on GD20) At MD, decrease pup b.w. in F1b. No effect on prenatal growth or viability in F2b Significant increase % malformed foetuses in F2b at LD (higher incidence of short rib, not considered treatment-rel.)	Klimisch: 2 Equivalent to OECD TG416. GLP study. Meets generally accepted scientific standards with acceptable restrictions. Test animals experienced decreased water consumption due to taste aversion. Animal room climate controls failed at one point during study (room t° 3-5° higher than normal and lower levels of humidity). Limitations: estrous cycle, sperm measures not conducted. NOAEL offspring toxicity not reliable.	RTI (1988a, b)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
3-generation study Oral (diet) Rat (Wistar) 30M + 30F/group (F0, F1b, F2b) ➔ after 11wk: 20F mated with 20M Ni sulphate hexahydrate ($NiSO_4 \cdot 6H_2O$ containing 22.3 % Ni) 0, 250, 500 and 1000 mg Ni/kg food = 0, 5, 50, 125 mg Ni/kg b.w. per day Litters: reduced to 10 offsprings on d5	Parental toxicity: 50 Reproductive toxicity: 125 Offspring toxicity: -	Parental toxicity: 125 Reproductive toxicity: - Offspring toxicity: 5	Parents: slight decrease b.w. at HD (8 % in F, 13 % in M). Higher incidence of stillborn in F1 (not observed in F2 or F3). Decrease nb. fetuses/litter at HD, dose-rel. decrease nb. weaning fetuses/litter. Decreased b.w. of weanlings at 1 000 mg Ni/kg food in all generations (recovery between weaning and subsequent mating). Histopathology of weanlings: no lesions.	Klimisch: 2 Limitations: lack of statistical analysis, reporting of results using pups rather than litters as the unit.	Ambrose et al. (1976)
3-generation study Oral (drinking water) Rat (Long Evans) 5 M + 5F/group 5 mg/L Ni salt (Diet contained 0.31 mg Ni/kg) Nb. litters: 11, 15 and 10 in F1, F2 and F3	-	5 mg Ni salt/L	F1: 9.1 % young deaths, and 30.6 % runts. F2: 10.2 % young deaths, and 5.1 % runts. F3: 21.0 % young deaths, and 6.2 % runts, few M born. The size of the litters decreased in F3.	Klimisch: 3 Very limited and poorly reported study	Schroeder and Mitchener (1971)
1-generation Oral (gavage) Rat (Sprague Dawley) 8M + 8F/group Ni sulphate hexahydrate 0, 10, 20, 30, 50 and 75 mg/kg b.w. per day = 0, 2.2, 4.4, 6.6, 11 and 17 mg Ni/kg b.w. per day	Parental and reproductive toxicity: 17 Offspring toxicity: 4	Parental and reproductive toxicity: - Offspring toxicity: 2.2	Parents: no effect on F0 survival, growth, mating behaviour, copulation, fertility, precoital intervals, gestation lengths or gross necropsy findings. Increase mean post-implantation loss at ≥ 30 mg/kg b.w. per day Pups: increase incidence of dead pups on LD 0 and decrease mean live litter size at HD (and lower mean live litter size than hist. C at ≥ 30 mg/kg b.w. per day). No effect on growth of surviving F1 pups during lactation, no effect on survival or growth of F1 pups from PND 22 for several weeks following weaning.	Klimisch: 2 Equivalent to OECD TG415. GLP study. Meets generally accepted scientific standards with acceptable restrictions.	SLI (2000a) Siglin (2000a)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
2-generation Oral (gavage) Rat (Sprague Dawley) F0 & F1: 28 rats/sex/group Ni sulphate hexahydrate 0, 1.0, 2.5, 5.0 and 10.0 mg/kg b.w. per day = 0, 0.2, 0.6, 1.1 and 2.2 mg Ni/kg b.w. per day	Parental and reproductive toxicity: 2.2 or 1.1 (EU RAR, 2008)	Parental, reproductive toxicity: - Offspring toxicity: - or 2.2 (EU RAR, 2008)	Parents: No effect on F0 or F1 survival, growth, mating behaviour, fertility, gestation, parturition or lactation. No treatment-related mortality or clinical signs of toxicity in F0 or F1 rats. No effect on estrous cycling, sperm parameters, copulation and fertility indices, precoital intervals, gestation lengths, gross necropsy findings or onset of sexual maturation in F1 rats. Pups: no effect on F1 or F2 pup viability and growth.	Klimisch: 2 Equivalent to OECD TG416. GLP study. Meets generally accepted scientific standards with acceptable restrictions. HDL did not result in toxicity of the parental animals.	SLI (2000b) Siglin (2000b)
1-generation/2-litter 11-week prior to mating + during 2 successive gestation + lactation periods Oral (drinking water) F Rat (Long Evans) 34 F Ni chloride 0, 10, 50 or 250 mg Ni/L = 0, 1.3, 6.8, 31.6 mg Ni/kg b.w. per day mated with M	Maternal toxicity: 1.3 Fertility: 31.6 Offspring toxicity: -	Maternal toxicity: 6.8 Fertility: - Offspring toxicity: 1.3	Dams: decrease water intake and increase food intake at HD. Decrease b.w. (6 %) on GD 21 at HD in G1, b.w. gain during G1 at 2 HD, small decrease in prolactin at HD No treatment-related effect on reproductive performance indices (mating success, rate of impregnation). Pups: decrease birth weight in M at MD during L1, no treatment-related effect on weight gain. Dose-rel. increase dead pups/litter, signif. at HD in L1 and at all doses in L2.	Klimisch: 2 Not a standard test method. Perinatal toxicity. Sperm quality and oestrus cyclicity not investigated.	Smith et al. (1993)
14-day Oral (drinking water) F Mouse (CD-1) Ni chloride 0, 1 000 mg/L		1 000 mg Ni chloride/L	Dams: decrease b.w. gain and water consumption, decrease TRH-stimulated release of prolactin during pregnancy. Pups: decrease number implantations sites and pups/litter No effect on pup b.w. at delivery.	Klimisch: 4 Abstract. 1 dose	Reynolds and Fail (1990)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
Oral (drinking water) Wistar rat 6M and/or 6F/group (9 groups) F: control F: 10, 30 mg or 100 $NiCl_2 \cdot 6H_2O$/kg b.w. per day 14 days before mating, mating, gestation and lactation = 2.47, 7.41 and 24.7 mg Ni/kg b.w. per day F: 30 mg $NiCl_2.6H_2O$ /kg b.w. per day 100 days before mating, mating, gestation and lactation = 7.41 mg Ni kg b.w. per day F: 100 $NiCl_2 \cdot 6H_2O$ /kg b.w. per day + 0.3 mg/L Se 14 days before mating, mating, gestation and lactation = 24.7 mg Ni/kg b.w. per day M: 30 mg $NiCl_2.6H_2O$/kg b.w. per day 28 days before mating = 7.41 mg Ni/kg b.w. per day M: 30 mg $NiCl_2 \cdot .6H_2O$/kg b.w. per day + 0.3 mg/L Se 28 days before mating = 7.41 mg Ni/kg b.w. per day M: 30 mg $NiCl_2. \cdot 6H_2O$/kg b.w. per day 42 days before mating = 7.41 mg Ni/kg b.w. per day M + F: M: 30 mg $NiCl_2. \cdot 6H_2O$/kg b.w. per day 28 days before mating and mating mated with F: 30 mg $NiCl_2. \cdot 6H_2O$/kg b.w. per day 28 days before mating, mating, gestation and lactation = 7.41 mg Ni/kg b.w. per day	Reproductive toxicity: -	Reproductive toxicity: 2.47	Breeding success: When M exposed for 28 days: decrease fertility index, no improvement by addition of Se. All F exposed groups: fertility index = 100 %. Slight decrease gestation index (not signif.) in F treated groups. Decrease gestation index when M treated for 28 days (stat. signif.) and slight decrease when M treated for 42 days or when M and F treated for 28 days. Decrease pup viability in HD F treated group and slight increase pup mortality in other F treated groups at weaning. Severe pup mortality in M treated group for 28 days, milder effect for 42 days. High pup mortality when both parents treated. Decrease proportion of M/litter in treated groups. Pups that died during lactation in treated groups were runts. Protective effect of Se. Pups: lower b.w., liver and kidney weight in F HD treated groups. Accumulation of Ni in kidneys > liver > skin of dams and pups from treated dams. Increase concentration of Ni in pups from HD F treated also with Se. Testis: Smaller mean diameters of seminiferous tubules, shrunk or even closed tubules in M exposed to Ni. Fewer basal spermatogonia in M exposed for 28 days, not for 42 days.	Klimisch: 2 No guideline followed. Effects on reproduction Low number of animals/group.	Käkelä et al. (1999)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
2-year study Oral (diet) Rat 25M + 25F Ni sulphate hexahydrate 0, 100, 1 000 and 2 500 mg Ni/kg food = 0, 5, 50 and 125 mg Ni/kg b.w. per day.	Systemic toxicity: 5 Reproductive toxicity: 125	Systemic toxicity: 50 Reproductive toxicity: -	No effect on reproductive organs.	Klimisch: 2 Limitations: 2-year survival was poor (particularly in controls and M HD), limited number of necropsies due to high mortality.	Ambrose et al. (1976)
2-year study Oral (diet) Dog 3M + 3F Ni sulphate hexahydrate 0, 100, 1 000 and 2 500 mg Ni/kg food = 0, 1.8, 18, 45 mg Ni/kg b.w. per day.	Systemic toxicity: 18 Reproductive toxicity: 45	Systemic toxicity: 45 Reproductive toxicity: -	No effects on reproductive organs.	Klimisch: 2	Ambrose et al. (1976)
13-week oral (drinking water) M Rat (Sprague Dawley) 8M/dose Nickel sulphate (NiSO4.6H2O) 0, 0.02, 0.05, 0.1% = 0, 44.7, 111.75, 223.5 mg Ni/L = 0, 4, 10 and 20 mg Ni/kg b.w. per day.	Systemic toxicity: 4 Reproductive toxicity: 20	Systemic toxicity: 10 Reproductive toxicity: -	Decrease testis weight in treated animals. No damages to the testes as verified by measurements of the activities of testicular enzymes (AP, acid phosphatase, lactate dehydrogenase). No gross or microscopic changes in any tissues examined.	Klimisch: 2 Limitations: only 8 animals/dose, histopathological evaluation not performed on all tissues, absence of electron microscopy	Obone et al. (1999)
91-day study Oral (gavage) Rat (Sprague Dawley) Ni chloride hexahydrate 0, 5, 35 or 100 mg Ni/kg b.w. per day	Systemic toxicity:- Reproduction toxicity: 100	Systemic toxicity:5 Reproduction toxicity: -	No histopathological alterations in reproductive tissues.	Klimisch: 2	American Biogenic Corporation (1988)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
35-day gavage (5 days/week) M albino Swiss mouse 20 M/dose Ni sulphate: 0, 5 and 10 mg/kg b.w. per day (0, 1.1 or 2.2 mg Ni/kg b.w. per day)	Systemic toxicity: 1.1 Reproductive toxicity: -	Systemic toxicity: 2.2 Reproductive toxicity: 1.1	No effect on b.w. gain, alopecia and sluggishness in M at HD. Decrease weight of testes, epididymis, seminal vesicles and prostate gland. Decrease sperm motility and sperm count (stat signif. at HD). Sperm abnormalities (head, neck and tail) in treated M. Alterations in the activities of marker testicular enzymes. At 10 mg Ni sulphate/kg b.w. per day: histopathological changes in testes (in seminiferous tubules: atrophy of centrally located tubules and disturbed spermatogenesis), epididymis (regressed epithelium and vacuolated cells) and seminal vesicles (reduction in size of vesicles in epithelium). Accumulation of Ni in epididymis > testes > seminal vesicles > prostate gland.	Klimisch: 2 Male reproductive effects. Only a limited number of parameters investigated: b.w. gain, male reproductive organ weights and sperm parameters. Only 6 males/group.	Pandey et al. (1999)
35-day gavage (5d/week) M albino Swiss mouse 6M/dose Ni sulphate: 0, 5, 10 or 20 mg/kg b.w. per day (0, 1.1 or 2.2 mg Ni/kg b.w. per day) or Ni chloride: 0, 5, 10 or 20 mg/kg b.w. per day (0, 1.2 or 2.5 mg Ni/kg b.w. per day).	Systemic toxicity: 1.1 Reproductive toxicity: -	Systemic toxicity: 2.2 Reproductive toxicity: 1.1	Dose-related decrease b.w. gain at 10 and 20 mg/kg b.w. per day Decrease weights (a, r) of testes, epididymis, seminal vesicles and prostate gland at 20 mg/kg b.w. per day Dose-related decrease in sperm motility and count at 10 and 20 mg/kg b.w. per day Dose-related and salt specific increase in abnormal sperm (head, neck and tail region) More marked spermatotoxic action of Ni chloride compare to Ni sulphate	Klimisch: 2 Male reproductive effects	Panday and Srivastava (2000)
6-month gavage (5d/week) M albino Swiss mouse 10M/dose Ni sulphate: 0, 20 mg /kg b.w. per day (0 or 2.2 mg Ni/kg b.w. per day).	Systemic and reproduction toxicity: -	Systemic and reproduction toxicity: 2.2 ay	Slight decrease b.w. in treated males, no outward signs of toxicity Decrease in normal (testosterone-dependent) proteinuria Testes: no effect on weight or histology Seminal vesicles: lower weight, smaller size (diameter), lower secretory activity of the cells of the vesicular epithelium. Accumulation of Ni in testis interstitial tissue.	Klimisch: 2 Male reproductive effects. Only 1 dose tested Limited number of parameters investigated	Pandey and Singh (2001)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
Single oral (gavage) Mouse Nb Control, 23 mg Ni/kg b.w. per day as Ni nitrate ($NiNO_2$ 72.2 mg/kg) 28 mg Ni/kg b.w. per day as Ni sulphate ($NiSO_4$ 73 mg/kg) 43 mg Ni/kg b.w. per day as Ni chloride ($NiCl_2$ 95 mg/kg)	-	23	Increase sperm head abnormalities in epididymes observed 5 weeks after the last exposure.	Klimisch: 3 Limited study: sperm head abnormalities, limited information reported.	Sobti and Gill (1989)
3-6-9- and 12-week oral (pellets) M mouse (ICR) 10M/group 10 mg Ni chloride/kg b.w.	-	10 mg $NiCl_2$/kg b.w.	No clinical signs of toxicity or mortality Time related effects on b.w. in all treated groups, signif. decrease only in treated group after 3 weeks Testis: time-related changes: - after 3 weeks: increases of empty spaces in the seminiferous epithelium; - from week 6 significant increases in % seminiferous tubules, degeneration of seminiferous epithelium with empty spaces in the epithelium, % tubule lumen, decreases in % interstitium. Germinal cells released into the tubule lumen; - after 9 week: in addition, decreases in blood vessels and increases in diameter of tubules. Release of dead epithelial cells into the tubular lumen, disintegration of the epithelium in some tubules. Decrease interstitial tissue relative volume.; - after 12 weeks: degeneration of seminiferous epithelium with necrotized germ cells releasing into the tubule lumen followed by increased occurrence of empty spaces in the tubules. Decrease relative volume of seminiferous epithelium, seminiferous tubules and diameter of the tubule.	Klimisch: 2 Effects of Ni on testis (morphometry, histopathology, stat. analysis).	Toman et al. (2012)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
Oral (diet) Weanling mice (Webster swiss) Ni acetate 0, 1 100 or 1 600 mg/kg food 1st exp: 4-week exposure 2d exp: Exposure: weaning, maturation and breeding	2d exp: Parental and reproductive toxicity: 1 600 mg Ni acetate/kg food Offspring toxicity: 1 100 mg Ni acetate/kg food	2d exp: Parental and reproductive toxicity: - Offspring toxicity: 1 600 mg Ni acetate/kg food	1st exp: decrease b.w. at HD in both sexes and at LD in F, decrease feed consumption in M. 2d exp: Parents: No effect on mature b.w. or on conception rate Pups: slight dose-rel. decrease number of pups born Decrease number of pups weaned at HD.	Klimisch: 3 Limited study.	Weber and Reid (1969)
Developmental toxicity					
35-day gavage (5 d/wk) M albino Swiss mouse 20M/dose Ni sulphate: 0, 10 mg/kg b.w. per day (0 or 2.2 mg Ni/kg b.w. per day) mated with untreated females (15 dams/dose).	-	2.210 2.2	Fertility index of exposed male mice 46.6 % compared to 66.6 % in controls. No effect on number of *corpora lutea*. Decrease number of pre- and post-implantations and increase number of resorptions. Decrease foetal weight.	Klimisch: 2 Male mediated developmental toxicity. Only one dose tested	Pandey et al. (1999)
GD 2-17 Oral (drinking water) F mouse (CD-1) Ni chloride 7 groups of 12 received 500 mg/L Ni in water and ordinary feed, 3 groups of 24 received 1 000 mg/L Ni in water and 100 mg/kg food in feed 0, 80, 160 mg Ni/kg b.w. per day	Developmental toxicity: 80	Developmental toxicity: 160	Dams: decrease b.w. at HD Pregnancy rate: 68 %, 65 % and 21 % in controls, LD and HD. Increase spontaneous abortions at high dose. Fetuses: No signif. effect on living, dead or total foetuses. Decrease fetal mass/litter at HD.	Klimisch: 3 Maternal toxicity observed; test substance not described, test substance not measured in drinking water, dosing strategies for each dose level differed. Only 2 doses. Not guideline compliant.	Berman and Rehnberg (1983)
GD 8-12 Oral (gavage) F mouse (28) (ICR/SIM) 0, 200 Ni chloride mg/kg b.w. per day = 0, 90.6 mg Ni/kg b.w. per day	Maternal toxicity: - Developmental toxicity: 90.6	Maternal toxicity: 90.6 Developmental toxicity: -	Dams: 1/28 death, decrease b.w. gain Neonates: no effect	Klimisch: 3 Screening test, only 1 dose, limited information reported A wide variety of substances were tested	Seidenberg et al. (1986)

Study Doses in mg Ni/kg b.w. per day	NOAEL	LOAEL	Effects	Study deficiencies	Reference
GD 8-12 Oral (gavage) Pregnant F mouse (CD-1) Ni chloride 0, 100 mg $NiCl_2$/kg b.w. per day 0, 45.3 mg Ni/kg b.w. per day C: 10 litters, Ni: 8 litters			Dams: No effect on % pregnant, age at parturition, litter size. Pups: No effect on number of live pups on day 3, b.w. on day 3, 22 or 30. Viability day 30: 81 % compared to 91 % in controls. No effect on male b.w., liver, testes, seminal vesicle or kidney weight at necropsy. At PND21, no effect on figure eight maze reactive locomotor activity levels.	Klimisch: 3 Very limited study and report Screening test on 36 substances 1 dose tested Observations: postnatal viability, growth, morphology, locomotor activity, reproductive function of the offsprings Short dosing period and limited behavioural testing	Gray and Kavlock (1984), Gray et al. (1986)
GD 6-13 Oral (gavage) F Swiss albino mouse (10/group) Ni chloride hexahydrate ($NiCl_2.\cdot 6H_2O$) 0, 46, 92 or 185 mg Ni/kg b.w. per day Sacrifice on day 18	Maternal toxicity: 46 Developmental toxicity: -	Maternal toxicity: 92 Developmental toxicity: 46	Dams: decrease feed and water consumption at 2 HD, dose-dependent decrease in b.w. (stat. signif at 2 HD), decrease nb. implant sites at 2 HD, non-signif. decrease placental weight in treated groups HD: decrease number live fetuses/dam, increase % postimplantation death, % resorptions, macerated and dead foetuses. Fetuses: dose-dependent decrease in b.w., increase fetal malformations mainly in 2 HD (hydrocephaly, open eyelids, microphthalmia, exophthalmia, club foot, umbilical hernia and skeletal anomalies), reduced ossification (nasal, frontal, parietal, intraparietal and supraoccipital bones, absence/gap between the ribs, reduced/fused sternebrae, vertebral centra and caudal vertebrae, reduced pelvic elements, absence of carpals, metacarpals, tarsals, metatarsals and phalanges).	Klimisch: 2	Saini et al. (2013)

2-GEN: 2-generation; AP: alkaline phosphatase; b.w. body weight; F: female; GD: gestation day; GLP: Good Laboratory Practice; HD: high dose; LD: low dose; LOAEL: lowest-observed-adverse-effect level; M: male; MD: mid dose; PND: post-natal day; Ni: nickel; NOAEL: no-observed-adverse-effect level; TRH: thyrotropin-releasing hormone.
(a): Klimisch score: 1 = RWoR: reliable without restriction, 2 = RWR: reliable with restriction, 3 = NR: non reliable, 4 = NA: non assignable. Dose conversion calculated by the CONTAM Panel.

Appendix G. Case report on toxicity of nickel in humans

Below are summaries of published reports on human health effects from intoxication by high amounts Ni in few or single cases after oral exposure or exposure via other routes (usually inhalation) where some digestion of Ni can not be excluded because of the high exposure. It should be noted that the exposure dose has not been quantified in all these cases.

Webster et al.(1980, also cited in Norseth, 1984) and WHO (2005) report nickel (Ni) intoxication in 23 haemodialysis patients where the dialysate was contaminated by leachate from a Ni-plated stainless steel water heater tank and who showed nausea, vomiting, headache, and weakness rapidly after exposure. Plasma Ni concentrations were about 3 mg/litre and persisted for 3–13 hours after dialysis.

Death following oral exposure to Ni was reported (Daldrup et al., 1983) for a 2-year-old child who accidentally ingested Ni sulfate crystals at a rough estimate of 570 mg Ni/kg). Four hours after ingestion, cardiac arrest occurred, and the child died 8 hours after exposure.

Death from adult respiratory distress syndrome was reported in one person who sprayed Ni using a metal arc process without wearing personal protective equipment. The death occurred 13 days after the 90-minute exposure; estimated concentration of 382 mg Ni/m^3 of principally metallic Ni (Rendall et al., 1994, see also Sundermann, 1993, ATSDR, 2005 and OEHHA, 2011). Histological examination of the lungs revealed alveolar wall damage and edema in alveolar spaces, and marked tubular necrosis was noted in the kidneys.

Fuentebella and Kerner (2010) present the case of a 13 year old boy with persistent nausea, vomiting, abdominal pain localized in the periumbilical area, weight loss and failure to thrive. Elevated Ni levels (28ng/mL) were found at a follow-up an appointment with his orthodontist who had two other patients with similar symptoms who were admitted to local hospitals. One of them had developed a localized reaction in his mouth from braces which prompted a Ni investigation. Removal of the braces leads to disappearance of the symptoms in all three cases. Four weeks after removal of the braces Ni levels were down to 0.7 ng/mL.

Phillips et al. (2010) re-examined a case report of a 38-year-old healthy male who inhaled nanoparticles of Ni while spraying Ni onto bushes for turbine bearings using a metal arc process for about 90 minutes and removing a protective advice. One day after he complained of cough, shortness of breath, and a tight chest, and four days after he was admitted to the hospital for tachypneic, pyrexial and cyanosed. He died after 13 days from acute respiratory distress syndrome. Ni nanoparticles (< 25 nm) were identified in lung macrophages using transmission electron microscopyHigh levels of Ni in urine were reported (780 µg/L) and his kidneys showed evidence of tubular necrosis. In addition, there was hematuria and proteinuria also indicative of kidney toxicity. The updated examination supports the idea that inhaled Ni can be absorbed systemically and affect other organs – see also OEHHA (2011).

Kunimasa et al. (2011) present the case of a 50-year-old man with a 30-year occupational history of welding presented with low-grade fever, fatigue and persistent dry cough who was diagnosed of having pneumonitis induced by inhalation of Ni fume after having inhaled Ni fumes 3 days previously at work.

Krecisz et al (2011) describe a non-atopic teenager with no body piercings with disseminated dermatitis manifesting itself as erythropapular lesions on his trunk and extremities. Six months prior to hospital admission, he had developed papular lesions in the periumbilical area at first wrongly associated with mechanical trauma caused by ametal buckle. Parents reported aggravation of skin changes after consumption of chocolate and other food products containing cocoa. When showing no response to treatment with topical corticosteroids and systemic antihistamines, one dose of parenteral bethametasone (Diprophos) was administered, and subsequently skin symptoms significantly diminished. Nevertheless, recurrence of widespread symmetric skin lesions was observed 1 day after

exposure to chocolate in his diet. Patch tests showed positive reactions to 1 % cobalt chloride (after 96 hours), 5 % Ni sulphate, 2 % copper sulphate, 2 % palladium chloride, and sesquiterpene lactone mix. Skin prick tests with common aeroallergens and cocoa were negative. Cocoa-specific IgE were not detected in serum.

Appendix H. Dose-response analysis using the Benchmark Dose Approach

H1. Methodology

This appendix outlines the methodology of the dose-response analysis applied for this opinion and reports details on the dose-response analysis using the BMD approach for the data on developmental and reproductive toxicity and on sensitized humans for the dose-response (DR) assessment of Nickel in Section 7.6. It details in particular the calculation of the BMD/L values by means of the BMDS software (version BMDSv2.4[15]) and PROAST (v26)[16] as reported in Section 7.6.

The quality of the dose-response data was checked by applying the criteria developed by EFSA (2009, 2011e) and used in previous opinions of the CONTAM Panel. According to that, modelling of dose-response data is considered poor, and therefore not informative, when at least one of the following criteria is met:

1. different accepted models result in widely different BMDL values;
2. the confidence interval around the BMD is wide;
3. the BMD is estimated by extrapolation considerably outside the range of observation, such that the BMD/L would depend heavily on the model used.

Since the BMDL is the lower 95 % one-sided confidence bound of the BMD and the BMDU is the upper 95 % confidence bound of the BMD, the interval BMDL/BMDU represents the 90 % confidence interval of the BMD. EFSA (2009) proposes as a general rule, that dose-response data should not result in a range of BMDL values from different accepted models that substantially exceed one order of magnitude and that the BMD/BMDL or the BMDU/BMDL ratio should not be considerably larger than by a factor of about 5 or 10, respectively. Furthermore, the BMDL should not be an order of magnitude higher respectively lower) than the highest (respectively lowest) applied dose level.

In general, when a model is extended by one or more parameters the resulting fit criterion may achieve a better goodness-of-fit of a model than with fewer parameters. However, it is unfavourable to use a model with too many parameters, since that may result in reduced precision of model predictions due to overfitting. Therefore, a formal criterion for model acceptance is needed to decide whether an extension in the number of parameters should be accepted or not. The goodness-of-fit at a statistical significance level has been suggested using the significance level of 5 % as default value and to examine all model fits for acceptability at the p-value of 0.05, preferably based on the (profile) maximum likelihood criterion. Deviations from using the 5 % significance level may be indicated when the power to detect a deviation from the model is high, e.g. when the sample size of the dose-response experiment is very large or when there is a large portion of the data in the low dose region e.g. when analysing human data collected in epidemiological studies

In some cases, not all models fit the dose-response data well and a BMDL may not be calculated (indicated below as of being not available – n.a.) for some models. This would indicate that the BMDL is very low and the model fit would be therefore unacceptable because of a too wide confidence interval. Occasionally, one observes non-convergence of the fitting algorithm indicated as not calculated (indicated below as n.c.).

When some models were excluded since they violated criteria 2 or 3 above the evaluation could be restricted to those models which complied with all three criteria, including so-called restricted models (also called constraints) which are available, in particular, in BMDS software where default restrictions can be chosen.

[15] US EPA: http://www.epa.gov/ncea/bmds/
[16] RIVM: http://www.rivm.nl/en/Documents_and_publications/Scientific/Models/PROAST

The tables below present the specific models fitted, log-likelihood values, characterisation of the model fit, BMD and BMDL values for the chosen BMR.

For dichotomous (quantal) data, all models available in BMDS software were selected for the BMD analysis using the default benchmark response (BMR) of 10 % extra risk as advised by the EFSA guidance on the use of benchmark dose (EFSA, 2009). The minimum BMDL obtained from all acceptable models was identified as the BMDL of that dataset as long as none of the above criteria was violated. Models allowing for restrictions were run only when the fit of the respective unrestricted models would not allow identifying an acceptable model and/or when restrictions would be indicated after inspection of the dose-response data.

For continuous data the best fitting model of the two nested families (Exponential and Hill) were identified using PROAST software and the minimum BMDL of the two families was chosen for characterising the dose-response data. For the benchmark response (BMR) the default value for continuous data recommended by EFSA (2009) of 5 % was used in the absence of statistical or toxicological considerations supporting a deviation from that default value, defined as a percent change of the magnitude of the response when compared to that predicted at background, i.e. a relative deviation from background. If not stated otherwise, the BMD analysis was based on summary data (means and standard deviations or standard errors, respectively) available from the studies. The nested character of the family of models (Exponential or Hill models) makes it possible to choose one best fitting model per family. In the PROAST software the appropriate model is automatically selected by consecutively fitting the members of the model family and choosing the model that cannot be statistically significant improved by a model having more parameters, when using a likelihood ratio test (Slob, 2002). Two sets of nested families, the Exponential (E) and the Hill (H) models were fitted to the data, respectively. The Exponential Model E1 denotes the reduced model for both families. Whereas the response level described by the Exponential models E2 and E3, and Hill models H2 and H3, respectively, tends to zero with increasing dose, it is allowed to tend to non-zero values (as sort of asymptotic saturation) in the Exponential models E4 and E5, and Hill models H4 and H5, respectively, thus allowing for an additional model parameter describing a positive response at high doses.

For interpreting the graphs and tables obtained by PROAST, it should be noted that the data of each dose group are assumed to be log-normally distributed and the software reconstructs from the summary data of (arithmetic) means and standard deviations a lognormal distribution by calculating the corresponding geometric means and geometric standard deviations Subsequently each model of the nested model family is fitted to these data and the fit is back-calculated to the original scale. It should be also noted that the graphs of PROAST software present the 95 % confidence interval of the means using the lognormal distribution such that the whiskers in the graphic do not indicate the range of the data or the range between plus/minus the standard deviation or standard errors of the mean but a 95 % confidence interval.

To account for the hierarchical type of data observed in reproductive and developmental studies and to address the presence of intra-litter correlations (denoted litter effects) and effects due to different litter sizes (modelled as covariate) nested models for dichotomous endpoints (with or without covariates) are an option to deal with such intra-litter correlations.. The endpoint analysed is then the incidence of a dichotomous (quantal) event (e.g. resorption, post-implantation loss, malformation) observed at each pup in each litter. This analysis requires that the dichotomous outcome is available for each pup which is not always possible e.g. when those data are summarized (may be also denoted as aggregated) as the number of pups per litter affected or as the ratio of the number of pups per litter affected over the total number of pups per litter per dose group. The total number of pups per litter of each dose group can in addition be considered as covariate or confounder in the dose-response analysis (litter specific covariate). This option was not used in this opinion since it has been argued to use a litter specific covariate with caution since it may erroneously indicate the presence of a litter size effect.

It should be noted that summarized data are aggregated on the level of the dam as the independent experimental unit and they characterize type and number of events occurring to the litter (e.g. the

incidence of resorption or malformations, or post-implantation losses). Thereby one assigns each dam a characteristic of her litter, e.g. presence of resorptions, the occurrence/incidence of post-implantation losses or the percentage of implementations losses. Important is the one-to-one relationship of the value of that endpoint to the individual dam such that these data could then be analysed either as dichotomous (quantal) or a continuous dose-response data using the approach described above.

Currently, the US EPA BMDS software is the best described commonly available computational tool to analyse reproductive and developmental data of the above described type. For individual hierarchical/nested data only three models are available in BMDS: the Nlogistic, the so-called NCTR, and the Rai-van-Ryzin (RvR) model. The first two models are very similar and differ only in be the shape of the model function (logistic versus Weibull function). Both use the beta-binomial distribution model to account for extra inter-litter variance of the portion of pups affected. Both models allow also for a litter specific covariate. The RvR model has been introduced to allow for a more general covariate that takes into account the condition of the dam before dosing. Without using this option it is identical to the NCTR model. Therefore, when applying for this opinion the results of NCTR and the RvR model were identical.

In contrast to usual dichotomous/quantal data (e.g. cancer incidence) or continuous data, no recommendations have been given for the choice of the BMR for hierarchical/nested reproductive and developmental data (EFSA, 2009; US EPA, 2012, 2014). It was argued by EFSA (2009) that for quantal data in developmental toxicity, the BMDL was on average closer to the average NOAEL for a BMR of 10 % than for a BMR of 5 % even if it was on average two-fold lower than the NOAEL. However, one should also note that when calculating a NOAEL for reproductive and developmental data the hierarchical type of data must be accounted in the same way as for dose-response modelling when choosing the statistical test method to derive a NOAEL, which is often not done or not sufficiently reported. Referring to the large developmental toxicity data base (Allen et al., 1994a, b; Faustman et al., 1994) it has been argued to choose a BMR = 5 % since the statistical power would be larger when a large number of offspring data can be used and developmental effects can be considered to be severe or frank. However, the type of hierarchical/nested data may also decrease statistical power due to intra-litter correlation implicating a lower effective sample size than the total number of pups and the recommended BMR = 10 % for cancer incidence is also based on frank effects.

For those reasons, in the absence of specific guidance on choosing a BMR and in the absence of specific statistical or toxicological arguments for deviating from the default BMR of 10 % recommended for dichotomous (quantal) data in general, the CONTAM Panel used a BMR = 10 % to derive a $BMDL_{10}$ as RP also for individual litter data from reproductive and developmental studies

H2. Benchmark Dose Analysis of developmental and reproductive studies in experimental animals

This section reports details of the BMD analyses of nested dichotomous, standard dichotomus and continuous dose-response data performed for the risk assessment of Ni in food. The first of the three subsections. Subsection H.2.1 reports the analysis of the summary data on the incidence of litters with post-implantation loss per treatment group, and the incidence of litters with three and more post-implantation losses per treatment group, observed in the multi-generation studies of SLI (2000a, b) in rats, see Table 16 in Sections 7.6.1 and 7.2.3. Since two dose-response datasets were available from a dose range finding study (DRF) and a main study (2-GEN) each endpoint was evaluated for

- DFR
- 2-GEN
- DRF and 2-GEN combined.

It should be remarked that the analysis was finally based on dose values in units of mg Ni/ kg b.w. per day with at maximum one significant digit after the decimal point i.e. 0.2, 0.6, 1.1, 2.2, 4.4, 6.6, 11, 17

mg Ni/kg b.w. per day. The results are organized in tables as recommendend by EFSA (2011e) usually with a graphic showing the fit of the model with the lowest BMDL(s).

The dose-response date of DFR, 2-GEN and DRF and 2-GEN combined were tested for a statistically significant increase of the incidence of litter with post-implantation loss using the Cochan-Armitage test for a (linear) trend (Piegorsch and Bailer, 1997). Whereas this trend was not statistically significant for the DRF and the 2-GEN study (P = 0.3 and P = 0.17 when using the exact version and not the chi-square approximation due to small sample sizes) it was significant for the pooled data DRF + 2-GEN (P = 0.00013). However, it should be noted that the power of the test was much higher for the pooled data since the sample size was larger and the dose range wider. Furthermore, the null model was tested versus the full model using the profile likelihoods calculated with the BMD analysis, see EFSA (2009). Note that an acceptable model should not be statistically significantly different from the full model, but the full model should be when compared with the null model.

Subsection H.2.2 reports on the analysis of the individual data of the multi-generation study of SLI (2000a, b) in rats of the post-implantation loss within the litters (where each pup in each litter is characterized by the presence or absence of an effect occurring between implantation and birth) using the Nlogistic, NCTR, and RvR model.

Subsection H2.3 reports on the analysis of quantal and quantitative summary of two studies analysed as supporting information using PROAST software. The analysis of data from the second generation of the multi-generation study of SLI (2000b) in rats is also reported.

H2.1. SLI Study (summary data)

H2.1.1. Dose range finding sub-study (DRF) of the SLI study

Table H1: BMD analysis of the incidence of litters with post-implantation loss per treatment group

incidence	2/8	5/8	6/8	6/7	7/7	8/8
dose	0	2.2	4.4	6.6	11	17 mg Ni/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} mg/kg b.w. per day	$BMDL_{10}$ mg/kg b.w. per day
Full model	na	6	17.16	–	–	–	–
Null model	na	1	26.40	-	-	-	-
Probit	na	2	17.46	0.92	yes	0.70	0.48
LogProbit	none	3	17.64	0.81	yes	0.94	0.029
	yes	2	s	s	s	s	0.37
Logistic	na	2	17.50	0.95	yes	0.68	0.43
LogLogistic	none	3	17.64	0.81	yes	0.95	0.029
	yes	2	s	s	s	s	0.37
Quantal-Linear	na	2	17.50	0.95	yes	0.36	0.22
Multistage Cancer	na	3	17.40	0.92	yes	0.48	0.23
Multistage	**none**	**2**	**17.40**	**0.92**	**yes**	**0.48**	**0.20**
Weibull	none	3	17.45	0.90	yes	0.57	0.0050
	Yes	2	s	s	s	s	0.22
Gamma	none	3	17.47	0.89	yes	0.56	0.00004
	yes	2				s	0.22

b.w.: body weight; na: not applicable; s: same as for unrestricted.

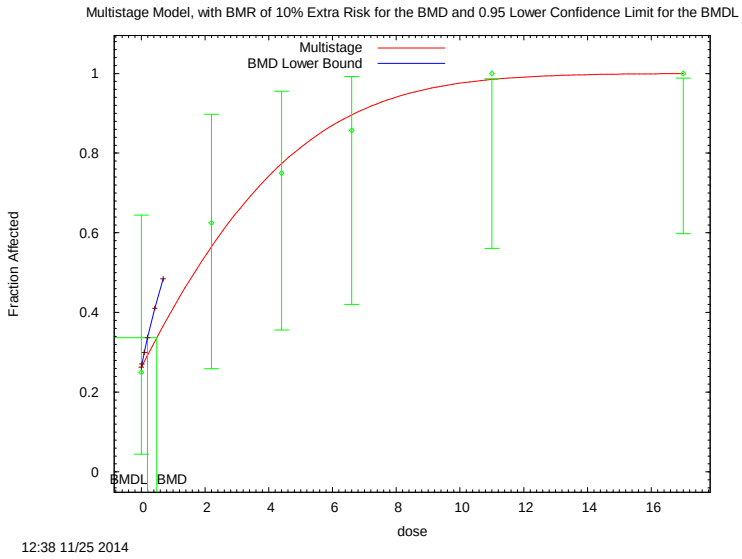


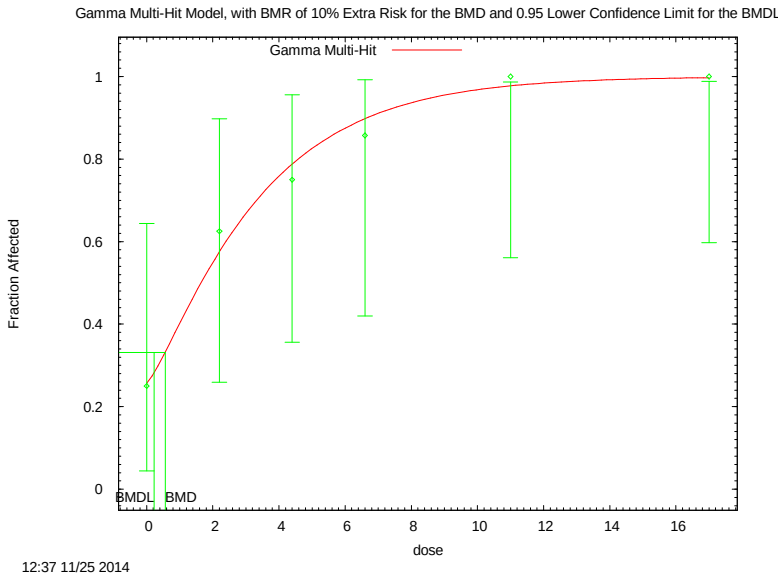
Table H2: BMD analysis of the incidence of litters with three or more post-implantation losses per treatment group

incidence	0/8	1/8	1/8	2/7	3/7	7/8
dose	0	2	4	7	11	17 mg Ni/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} mg/kg b.w. per day**	**$BMDL_{10}$ mg/kg b.w. per day**
Full model	na	6	18.01	–	–	–	–
Null model	na	1	28.27	-	-	-	-
Probit	na	2	18.59	0.89	yes	4.17	2.80
LogProbit	none	3	19.22	0.66	yes	3.00	1.09
Logistic	na	2	18.65	0.87	yes	4.50	2.99
LogLogistic	none	3	19.06	0.72	yes	3.15	1.07
Quantal-Linear	na	2	19.46	0.72	yes	1.60	1.05
Multistage Cancer	na	3	18.54	0.90	yes	2.96	1.25
Multistage	none	2	18.54	0.90	yes	2.96	1.25
Weibull	none	3	18.68	0.85	yes	3.13	1.03
Gamma	**none**	**3**	**18.83**	**0.80**	**yes**	**3.02**	**0.85**

b.w.: body weight; na: not applicable.



H2.1.2. Two generation substudy (2-GEN) of the SLI study

Table H3: BMD analysis of the incidence of litters with post-implantation loss per treatment group

incidence	13/25	18/26	15/25	19/26	19/28
dose	0	0.2	0.6	1.1	2.2 mg/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} mg/kg b.w. per day**	**$BMDL_{10}$ mg/kg b.w. per day**
Full model	na	5	82.91	–	–	–	–
Null (reduced) model	na	1	84.47	-	-	-	-
Probit	na	2	84.06	0.51	yes	0.81	0.32
LogProbit	none	3	83.42	0.60	yes	$<10^{-9}$	failed
	yes	2	s	s	s	s	S
Logistic	na	2	84.05	0.51	yes	0.79	0.31
LogLogistic	none	3	83.41	0.52	yes	$<10^{-10}$	failed
	yes	2	s	s	s	s	s
Quantal-Linear	na	2	84.04	0.52	yes	0.72	0.25
Multistage Cancer	**na**	**2**	**84.04**	**0.45**	**yes**	**0.72**	**0.22**
Multistage	none	2	83.70	0.45	yes	0.19	0.068
	yes	2	s	s	s	0.72	0.25
Weibull	none	3	84.04	0.52	yes	$<10^{-11}$	failed
	yes	s	s	s	s	0.72	0.25
Gamma	none	3	failed	na	na	na	na

b.w.: body weight; failed: Benchmark dose computation failed. Lower limit includes zero; if: invalid fit; na: not applicable; s: same as for unrestricted.

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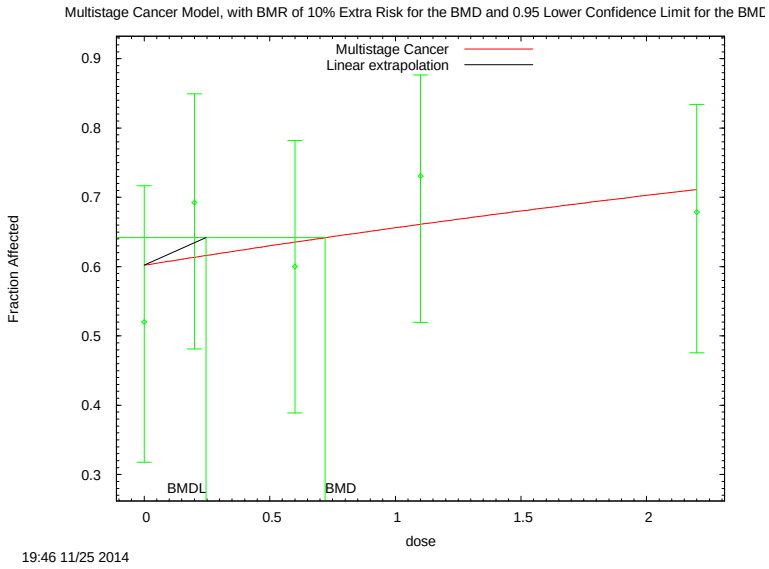


Table H4: BMD analysis of the incidence of litters with three or more post-implantation losses per treatment group

incidence	3/25	3/26	5/25	5/26	9/28
dose	0	0.2	0.6	1.1	2.2 mg/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} mg/kg b.w. per day**	**$BMDL_{10}$ mg/kg b.w. per day**
Full model	na	5	61.29	–	–	–	–
Null (reduced) model	na	1	63.64	-	-	-	-
Probit	na	2	61.46	0.95	yes	1.12	0.74
LogProbit	none	3	61.46	0.85	yes	0.94	0.019
LogProbit-Restrict	yes	2	61.58	0.90	yes	1.30	0.79
Logistic	na	2	61.47	0.95	yes	1.16	0.78
LogLogistic	none	3	61.46	0.85	yes	0.97	0.017
	yes	2	s	s	s	s	0.41
Quantal-Linear	na	2	61.46	0.95	yes	0.91	0.48
Multistage Cancer	na	3	61.45	0.85	yes	0.99	0.48
Multistage	**none**	**3**	**61.45**	**0.85**	**yes**	**0.99**	**0.28**
Weibull	none	3	61.45	0.85	yes	0.98	0.016
	yes	2	s	s	s	s	0.48
Gamma	none	3	61.45	0.85	yes	0.98	0.016
	yes	2	s	s	s	s	0.48

b.w.: body weight; if: invalid fit; na: not applicable; s: same as for unrestricted.

H2.1.3. DRF and 2-GEN of the SLI study combined

Table H5: BMD analysis of the incidence of litters with post-implantation loss per treatment group

incidence	15/33	18/26	15/25	19/26	24/36	6/8	6/7	7/7	8/8
dose	0	0.2	0.6	1.1	2.2	4.4	6.6	11	17 mg/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} mg/kg b.w. per day**	**$BMDL_{10}$ mg/kg b.w. per day**
Full model	na	9	101.04	–	–	–	–
Null (reduced) model	na	1	111.56	-	-	-	-
Probit	na	2	103.52	0.66	yes	0.69	0.45
LogProbit	none	3	104.18	0.39	yes	3.34	0.0003
	yes	2	s	s	s	s	0.57
Logistic	na	2	103.66	0.64	yes	0.64	0.41
LogLogistic	none	3	104.20	0.39	yes	2.96	0.0002
	yes	2	s	s	s	s	0.14
Quantal-Linear	na	2	103.73	0.61	yes	050	0.29
Multistage Cancer	na	3	103.54	0.54	yes	0.76	0.30
Multistage	**none**	**3**	**103.54**	**0.54**	**yes**	**0.76**	**0.28**
Weibull	none	3	103.73	0.50	yes	0.66	0.0002
	yes	2	s	s	s	s	0.29
Gamma	none	3	103.59	0.53	yes	0.089	$<10^{-5}$
	Yes	2	s	s	s	0.50	0.29

b.w.: body weight; if: invalid fit; na: not applicable; s: same as for unrestricted.

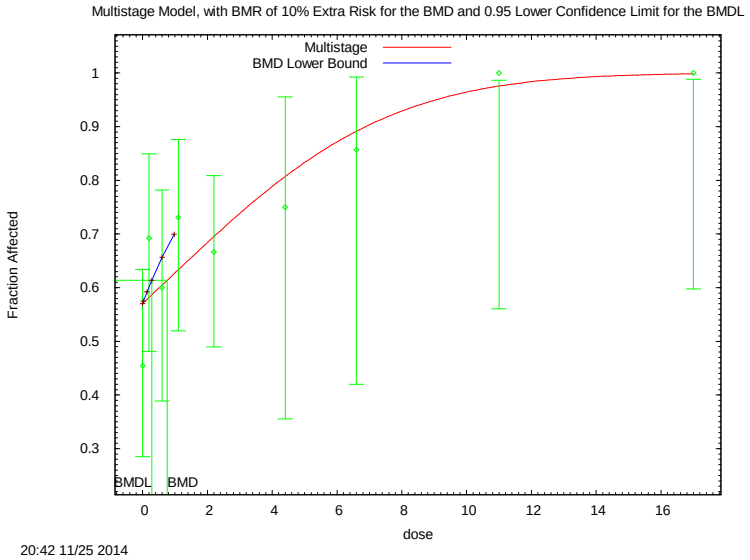


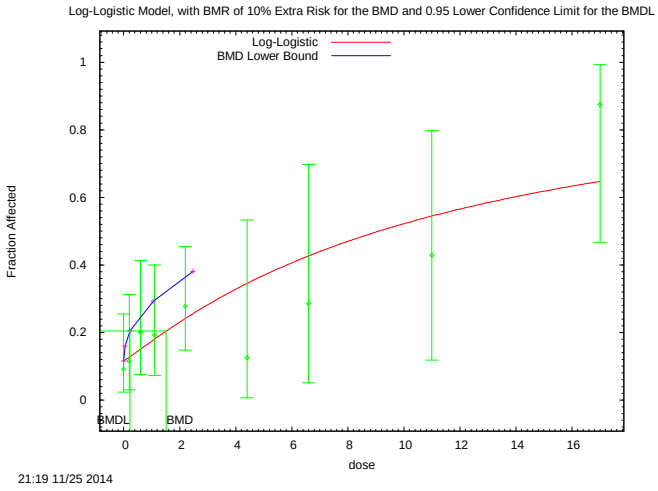
Table H6: BMD analysis of the incidence of litters with three or more post-implantation losses per treatment group

incidence	3/33	3/26	5/25	5/26	10/36	1/8	2/7	3/7	7/8
dose	0	0.2	0.6	1.1	2.2	4.4	6.6	11	17mg/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} mg/kg b.w. per day**	**$BMDL_{10}$ mg/kg b.w. per day**
Full model	na	5	80-86	–	–	–	–
Null (reduced) model	na	1	93.09	-	-	-	-
Probit	na	2	83.21	0.70	yes	2.93	2.18
LogProbit	none	3	**84.10**	**0.37**	yes	**1.38**	**0.22**
	yes	2	84.32	0.44	yes	3.14	1.93
Logistic	na	2	83.24	0.69	yes	3.13	2.29
LogLogistic	none	3	**83.91**	**0.41**	**yes**	**1.52**	**0.22**
	yes	2	s	s	s	s	0.73
Quantal-Linear	na	2	83.56	0.61	yes	1.65	1.03
Multistage Cancer	na	3	83.30	0.56	yes	2.50	1.07
Multistage	none	3	83.30	0.56	yes	2.50	0.97
Weibull	none	3	83.54	0.50	yes	7.05	0.24
	yes	2	s	s	s	s	1.03
Gamma	none	3	**83.54**	**0.50**	**yes**	**1.33**	**0.19**

b.w.: body weight; if: invalid fit; na: not applicable; s: same as for unrestricted.



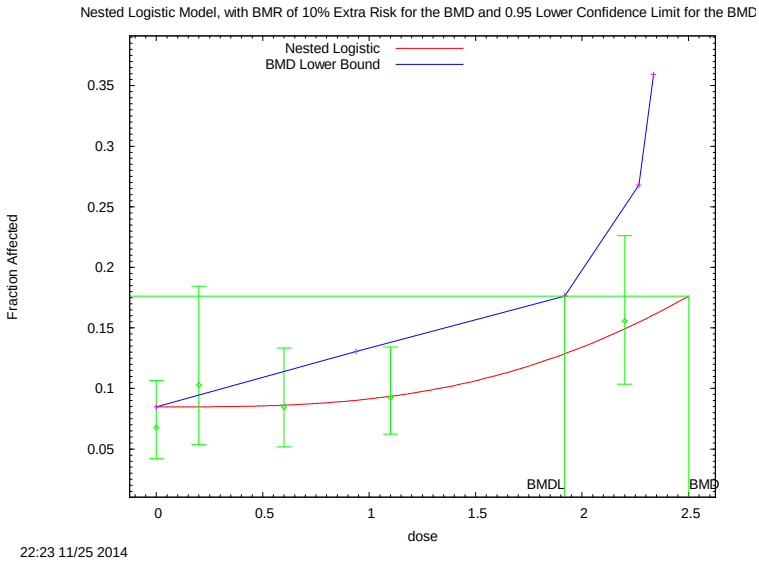
H2.2. SLI study – Individual data

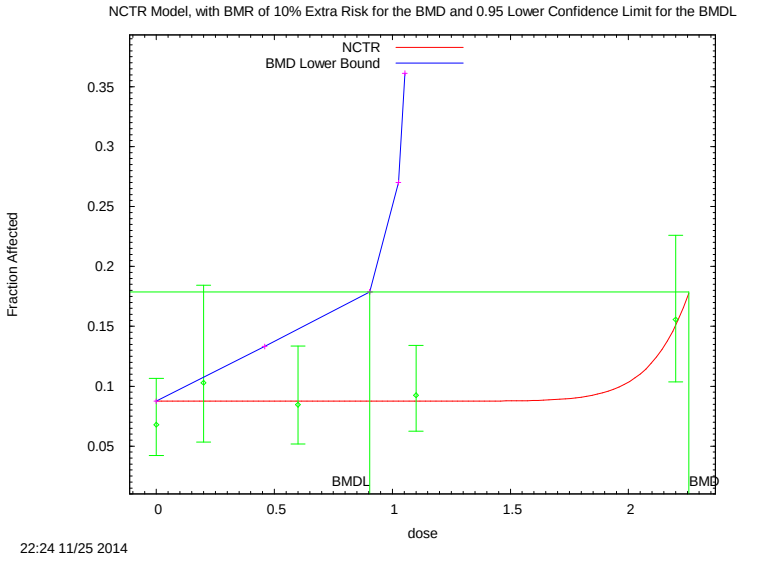
The DRF, the 2-GEN data and the combined DRF/2-GEN data were analysed by the available three nested dichotomous models NLogist, NCTR, and RvR using the 'Overall Mean' for fixed litter size. The 'Control Group Mean' was used only for some sensitivity considerations (not reported). The litter specific covariate was not used except for some sensitivity considerations (not reported). All analyses reported here accounted for intra-litter correlations. For some sensitivity considerations also analyses without accounting for litter effects were performed (not reported). For analysing the sensitivity of the BMD/L values on the usage of the DRF data the combined data were analyses by stepwise 'stripping off' the high dose data of the DRF part in order to check if there would be an undue strong influence of the high doses on the value of the BMD/L values in the combined analysis (not reported).

Only three models are available in BMDS, the so-called Nlogistic NCTR, and the Rai-van Ryzin (RvR) model, for details see US EPA (2012, 2014) and Allen et al. (1994b). In contrast to standard dichotomous/quantal data (e.g. cancer incidence) no recommendations have been given for the choice of the BMR for developmental data (EFSA, 2009; US EPA, 2012, 2014). In the absence of specific guidance on choosing a BMR and in the absence of specific statistical or toxicological arguments preferring a specific BMR, the CONTAM Panel used a BMR = 10 % to derive an RP for individual litter data available for the multi-generation study of SLI (2000a, b).

H2.2.1. DRF data of the SLI study

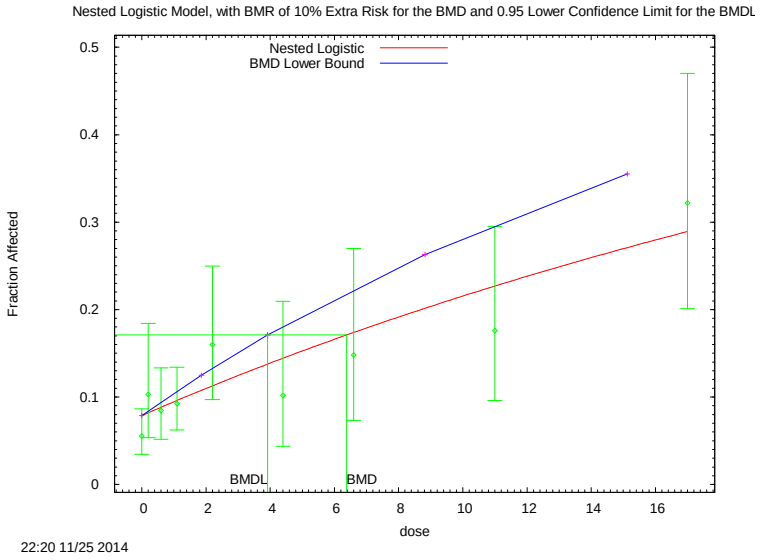
Model	model fit chi-square p-value	BMD_{10}	$BMDL_{10}$
Nlogist	0.0015	4.90	3.32
NCTR/RvR	0.0012	5.27	2.64

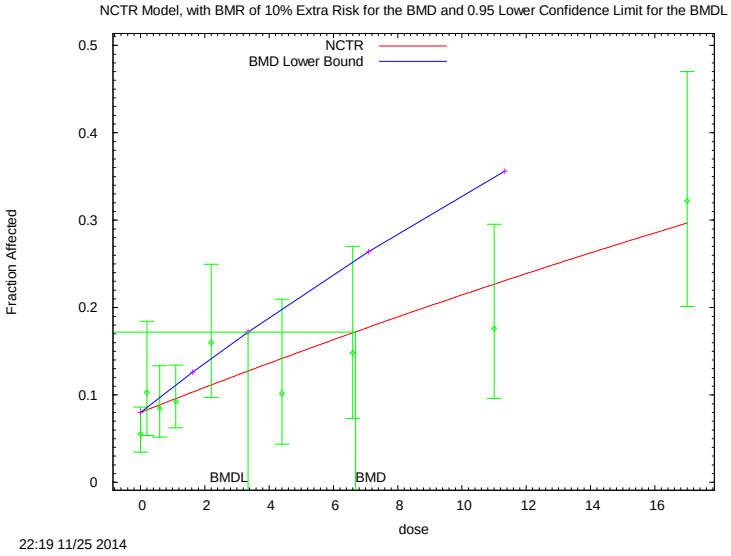

Nested Logistic Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMD
Nested Logistic
BMD Lower Bound
Fraction Affected
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.5
1
1.5
2
2.5
BMDL
BMD
dose
22:23 11/25 2014


NCTR Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL
NCTR
BMD Lower Bound
Fraction Affected
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.5
1
1.5
2
BMDL
BMD
dose
22:24 11/25 2014

H2.2.2. 2- GEN data of the SLI study

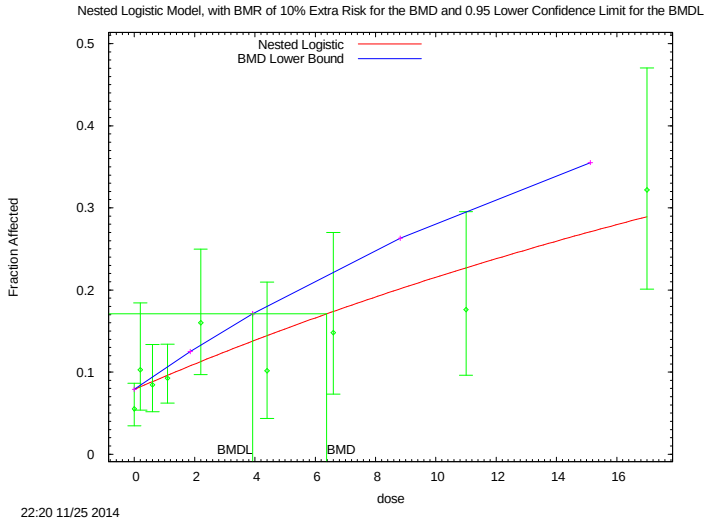
Model	model fit chi-square p-value	BMD_{10}	$BMDL_{10}$
Nlogist	0.028	2.50	1.91
NCTR/RvR	0.027	2.49	0.87

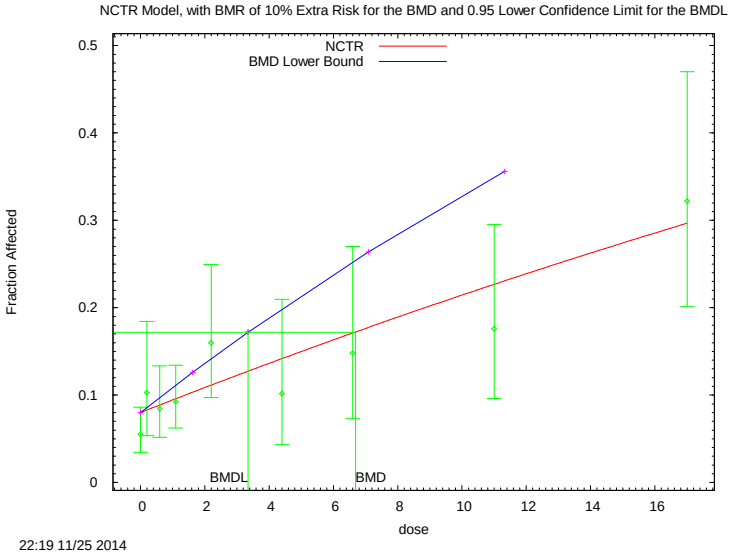




H2.2.3. DRF and 2- GEN data combined of the SLI study

Model	model fit chi-square p-value	BMD_{10}	$BMDL_{10}$
Nlogist	0.07	6.38	0 .92
NCTR/RvR	0.07	6.69	3.34



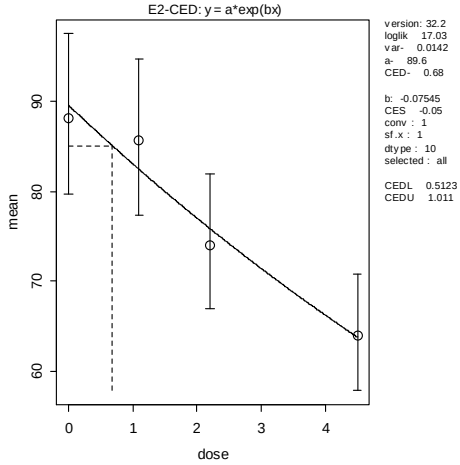


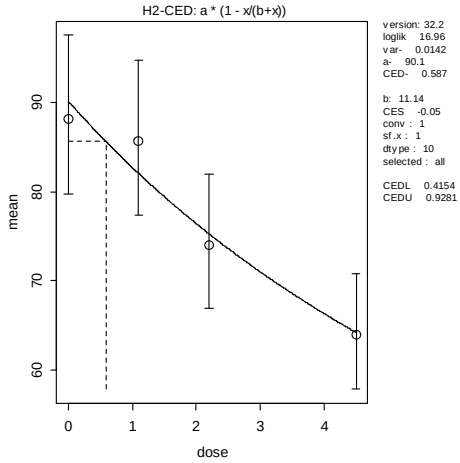
H2.3. Analysis of supporting dose response data of two studies in mice and rats

The quantitative data were analysed with PROAST. For study details see Section 7.2.3.

Table H7: Study of Pandey and Srivastava (2000) on nickel sulphate

a) percent motile sperms			
Nested model family	model selected	BMD_{05}	$BMDL_{05}$
Exponential	m2	0.68	0.51
Hill	m2	0.59	0.42

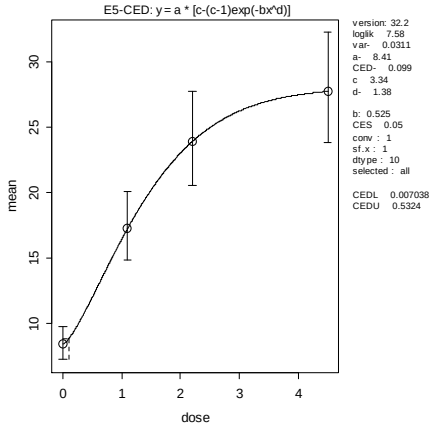


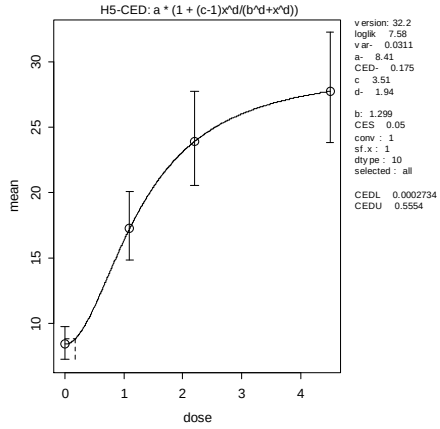


b) sperm count epididymis (x 10^7)			
Nested model family	model selected	BMD_{05}	$BMDL_{05}$
Exponential	m2	0.70	0.55
Hill	m2	0.62	0.46

c) sperm abnormalities
Standard deviations were approximated by reading them from the graphic.

Nested model family	model selected	BMD_{05}	$BMDL_{05}$
Exponential	m5	0.10	0.007
Hill	m2	0.18	0.0003



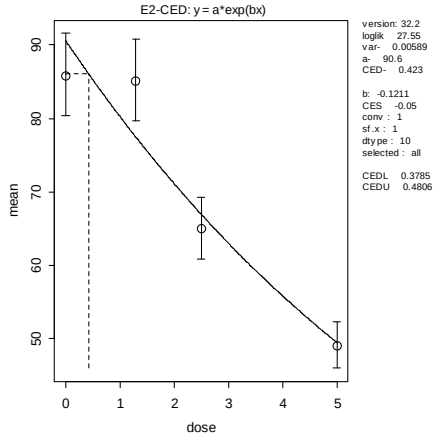


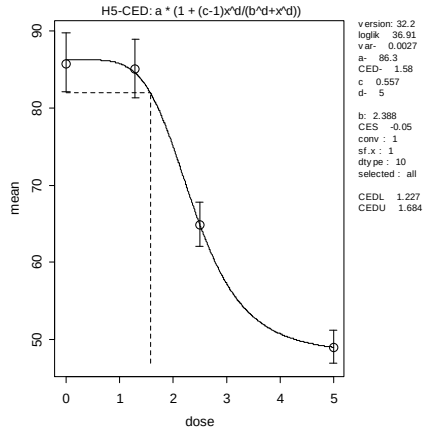
H2.3.2. Study of Pandey and Srivastava (2000) on Nickel chloride in mice

Table H8: Study of Pandey and Srivastava (2000) on nickel chloride in mice

a) percent motile sperms			
Nested model family	model selected	BMD_{05}	$BMDL_{05}$
Exponential	m2	0.42	0.38
Hill	m5	1.6	1.2

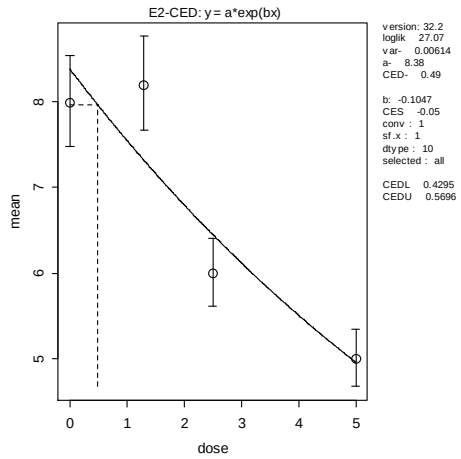
For the exponential family the full model is statistically significant better than selected model (P <0.0001).

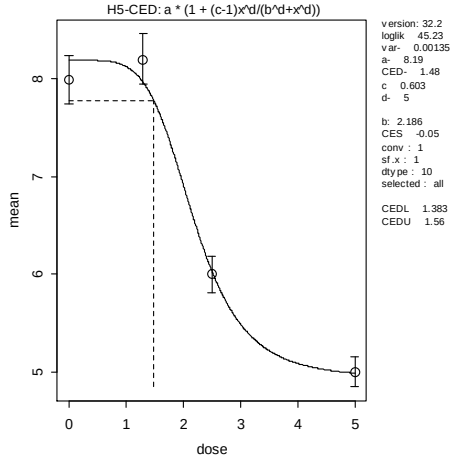




b) sperm count epididymis (x 10^7)			
Nested model family	model selected	BMD_{05}	$BMDL_{05}$
Exponential	m2	0.49	0.43
Hill	m5	1.5	1.4

The full model is statistically significant better than selected model (P $<10^{-9}$ and 0.005 for the exponential and the Hill model family, respectively).





H2.3.3. Study of Smith et al. (1993) on nickel chloride in rats

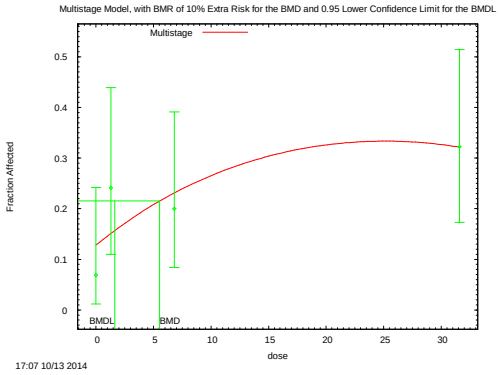
Numbers of litters with dead pups at birth (see Section 7.2.3) were analysed as quantal data. Only the 2nd breeding data showed a clear dose response pattern. The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit are shown in the Table H9. The lowest $BMDL_{10}$ of the unrestricted acceptable models was 1.6 mg Ni/kg b.w. (indicated in bold).

Table H9: BMD Analyis of incidence of dead pups at 2nd breeding

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg Ni/kg b.w per day)	$BMDL_{10}$ (mg Ni/kg b.w per day)
Full model	na	4	57.81	–	–	–	–
Null (reduced) model	na	1	61.17	-	-	-	-
Probit	na	2	59.45	0.19	yes	17.0	10.2
LogProbit	none	3	58.17	0.40	yes	0.09	failed
LogProbit-Restrict	yes	2	59.64	0.16	yes	21.1	12.0
Logistic	na	2	59.46	0.19	yes	17.5	10.8
LogLogistic	none	3	58.16	0.40	yes	0.08	failed
LogLogistic-Restrict	yes	2	59.35	0.21	yes	12.6	5.2
Quantal-Linear	na	2	59.40	0.21	yes	13.8	6.5
Multistage Cancer	na	2	59.40	0.21	yes	13.8	6.5
Multistage	**none**	**3**	**59.23**	**0.09**	**yes**	**5.5**	**1.6**
Weibull	none	3	58.15	0.41	yes	0.08	failed
Weibull-Restrict	yes	2	59.40	0.21	yes	13.8	6.5
Gamma	none	3	58.15	0.41	yes	0.07	failed
Gamma-Restrict	yes	2	59.39	0.21	yes	13.8	6.5

b.w.: body weight; failed: Benchmark dose computation failed. Lower limit includes zero; if: invalid fit; na: not applicable.



H2.3.4. F2 Generation of the SLI study

Table H10: BMD analysis of the incidence of litters with post-implantation loss per treatment group

incidence	13/24	18/26	16/25	18/23	14/24
dose	0	0.2	0.6	1.1	2.2 mg/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg Ni/kg b.w per day)	$BMDL_{10}$ (mg Ni/kg b.w per day)
Full model	na	5	77.28	–	–	–	–
Null (reduced) model	na	1	79.17	-	-	-	-
Probit	na	2	79.17	0.29	yes	14.3	0.44
LogProbit	none	failed	na	na	na	na	na
	yes	2	79.17	0.29	yes	94	0.76
Logistic	na	2	79.17	0.29	yes	18.4	0.42
LogLogistic	none	failed	na	na	na	na	na
Quantal-Linear	**na**	**2**	**17.19**	**0.29**	**yes**	**17.3**	**0.36**
Multistage Cancer	none	failed	na	na	na	na	na
Multistage	none	3	77.91	0.53	yes	0.12	0.054
Weibull	none	failed	na	na	na	na	na
Gamma	none	failed	na	na	na	na	na

b.w.: body weight; failed: Benchmark dose computation failed. Lower limit includes zero; if: invalid fit; na: not applicable; s: same as for unrestricted

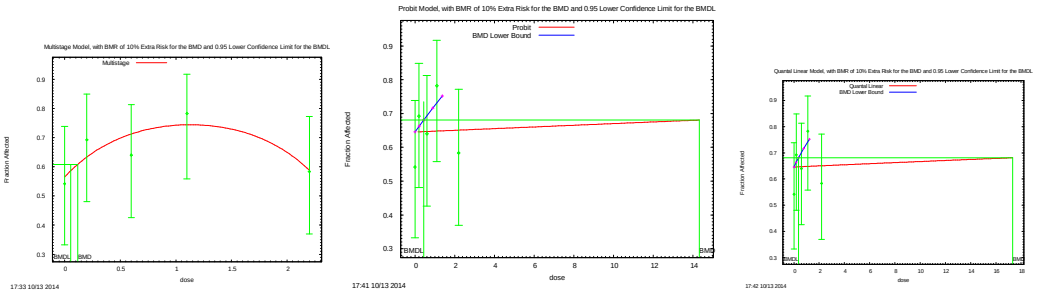


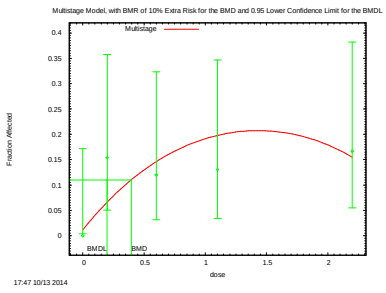
Table H11: BMD analysis of the incidence of litters with with 3 or more post-implantation loss per treatment group

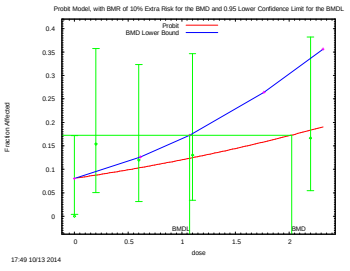
incidence	0/24	4/26	3/25	3/23	4/24
dose	0	0.2	0.6	1.1	2.2 mg/kg b.w. per day

The benchmark dose BMD_{10}, the 95 % benchmark dose lower confidence limit $BMDL_{10}$ values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed for an unrestricted model, also the respective restricted model using BMDS-Software default values were calculated. The result with lowest $BMDL_{10}$ of accepted models is given in bold for the unrestricted or the restricted models, in case when no acceptable restricted model was available.

Models	**Restriction**	**N of parameters**	**Minus Log-likelihood**	**P-value**	**Accepted**	**BMD_{10} (mg Ni/kg b.w per day)**	**$BMDL_{10}$ (mg Ni/kg b.w per day)**
Full model	na	5	40.05	–	–	–	–
Null (reduced) model	na	1	43.47	-	-	-	-
Probit	na	2	42.73	0.15	yes	2.02	1.07
LogProbit	none	3	40.19	0.97	yes	$<10^{-7}$	failed
	yes	2	43.00	0.12	yes	2.31	1.15
Logistic	na	2	42.75	0.15	yes	2.07	1.14
LogLogistic	none	3	40.19	0.97	yes	$<10^{-6}$	failed
	yes	2	na	na	na	na	na
Quantal-Linear	na	2	42.61	0.16	yes	1.65	0.65
Multistage Cancer	na	3	42.61	0.16	yes	1.65	0.65
Multistage	none	3	41.97	0.15	yes	0.40	**0.20**
Weibull	none	3	40.19	0.97	yes	<10-6	failed
	yes	2	48.98	0.42	yes	3.06	0.85
Gamma	none	failed	na	na	na	na	na
	yes	failed	na	na	na	na	na

b.w.: body weight; failed: Benchmark dose computation failed. Lower limit includes zero; if: invalid fit; na: not applicable; s: same as for unrestricted





H3. Benchmark Dose analysis for acte effects in sensitized humans

A total of 17 studies were included in the meta-analysis of Jensen et al. (2006), numbered in the following as in this paper from No 4 to No 20, corresponding also the numbers in the list of references of Jensen et al. (2006). Relevant information on and suitability for dose-response analysis for each study is noted below. Jensen et al. (2006) included in their dose-response analysis 9/17 studies in the three classes of 0.22 mg, 0.35 mg and 0.53 mg Ni as follows: No 12, 19; No 5, 6, 7, 11, 13; No 4, 14:

No 4: n = 12 females of 21–60 years, 5.6 mg Ni in 25 mg Ni sulphate once in one sequence
9/12 (75.0 %) positive reactions
not suitable

No 5: n = 28 (26 females, 2 males), 2.5 mg Ni as Ni sulphate
46.4 % positive reaction in those without placebo reaction
not suitable

No 6: n = 13 using 0 ,0.6, 1.2 , 2.5 mg Ni as Ni sulphate insufficient information on Ni exposure is given,
no meaningful dose response data

No 7: n=16, 2.5 mg Ni as Ni sulphate
4/13 (30.8 %) reactions to Ni
not suitable

No 8: n = 10 female, 0.5 mg Ni
6/10 females exhibited flares when exposed
not suitable

No 9: 16 female 0.6, 1.25, 2.5 mg Ni, 5 patients per dose
clear dose response observed for worsening of hand eczema: 2/5, 3/5, 5/5
used for dose-response analysis

No 10: n = 22 (Paper in Italian) 0.5 mg Ni per day, 2 days per week, 2 weeks, once repeated
reactions after 2 weeks (2 mg Ni total): 9/22 (40.9)
reactions after 4 weeks (4 mg Ni total): 8/22 (36.4)
not suitable

No 11: n=538, data from a challenge test with 2.24 mg Ni
240/538 (44.6) with response, and 31/49 (63 %) a second time
not suitable

No 12: no original data are reported
not suitable

No 13: n = 26 (24 female, 2 male; 19-67 years)
0.4 (n = 10), 2.5 (n = 10), 5.6 (n = 6) mg elemental Ni given in the form of $NiSO_4 \cdot 7H_2O$
5/10 , 5/ 10 and 6/6 with reaction after Ni only in the proportions of 3/10, 2/10 and 4/6; a positive response was defined as accentuation of previously noted physical signs (usually worsening of microvesicular hand eczema) or development of new physical signs (e.g. eczematous or erythematous eruptions).
no clear dose response and unusual endpoint

No 14: n = 19 female patients with nickel eczema, 2.5 mg Ni
8/19 (42.1 %) responder
not suitable

No 15: n = 146 cases; 2.5 mg Ni as Ni sulphate
n = 131 with positive patch test to Ni and/or Cobalt, 97 /131 to Ni alone.
inconsistent report
not suitable

No 16: n=25 female, n=22 patch test positive, 10 mg $NiSO_4$
22/25 (88.0 %) responded
not suitable

No 17: n = 28 (also on gold and combinations of gold and Ni), 2.5 mg Ni
response 3/9
not suitable

No 18: n = 20 sensitized female with vesicular hand eczema of pompholyx type, 12 µgNi/kg b.w.
response with flare-up symptoms 9/20 (45.0 %)
not suitable

No 19: n = 30 randomized to 0, 1, 3 mg Ni with 10 per group
flare-up reactions in 0/10, 2/10 and 9/9
clear DR

No 20: n = 40, randomized to 0, 0.3, 1, 4 mg Ni (10/dose)
clinical reactions: 1/10, 4/10, 4/10, 7/10
flare –up of previous sites of dermatitis: 1/10, 4/10, 4/10,6/10
flare –up former hand eczema: 1/5, 2/7, 2/8, 3/5
clear DR

The following three tables show the results of the BMD analysis when using mg Ni as dose. For the conversion to µgNi/kg b.w. see Section 7.6.2.

Table H12: Study No 13: 0.4 (n = 10) , 2.5 (n = 10), 5.6 (n = 6) mg nickel given as $NiSO_4 \cdot 7 H_2O$. Reactions were 5/10 , 5/ 10 and 6/6 for the 3 expousre groups, respectively.

The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed the restricted models using BMDS-Software default values were calculated. The results with lowest $BMDL_{10}$ of unrestricted models are given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg Ni)	$BMDL_{10}$ (mg Ni)
Full model	na	3	13.86	–	–	–	–
Null (reduced) model	na	1	17.32	-	-	-	-
Probit	na	2	15.11	0.11	yes	0.54	0.33
LogProbit	none	2	no fit	na	na	na	na
Logistic	na	2	15.21	0.10	yes	0.55	0.32
LogLogistic	none	2	13.86	0.98	yes	2.92	1.08
Quantal-Linear	**na**	**2**	**15.53**	**0.07**	**yes**	**0.41**	**0.18**
Multistage Cancer	na	2	14.67	0.20	yes	1.22	0.24
Multistage	none	2	no fit	na	na	na	na
Weibull	none	2	no fit	na	na	na	na
Gamma	none	2	13.93	0.71	yes	2.47	0.73

b.w.: body weight; na: not applicable.

Table H13: Study No 19: 0, 1, 3 mg Ni with 10 per group flare-up reactions in 0/10, 2/10 and 9/9

The benchmark dose (BMD_{-10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed the restricted models using BMDS-Software default values were calculated. The results with lowest $BMDL_{-10}$ of unrestricted models are given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg Ni)	$BMDL_{10}$ (mg Ni)
Full model	na	3	5.00	–	–	–	–
Null (reduced) model	na	1	19.25	-	-	-	-
Probit	na	2	5.00	1	yes	0.91	0.43
LogProbit	none	2	5.00	1	yes	0.93	0.55
Logistic	na	2	5.00	1	yes	0.95	0.44
LogLogistic	none	2	5.00	1	yes	0.96	0.55
Quantal-Linear	**na**	**2**	**8.02**	**0.05**	**yes**	**0.18**	**0.11**
Multistage Cancer	na	2	5.63	0.53	yes	0.54	0.22
Multistage	none	2	5.00	1	yes	0.96	0.31
Weibull	none	2	5.00	1	yes	0.82	0.38
Gamma	none	3	5.00	1	yes	0.82	0.45

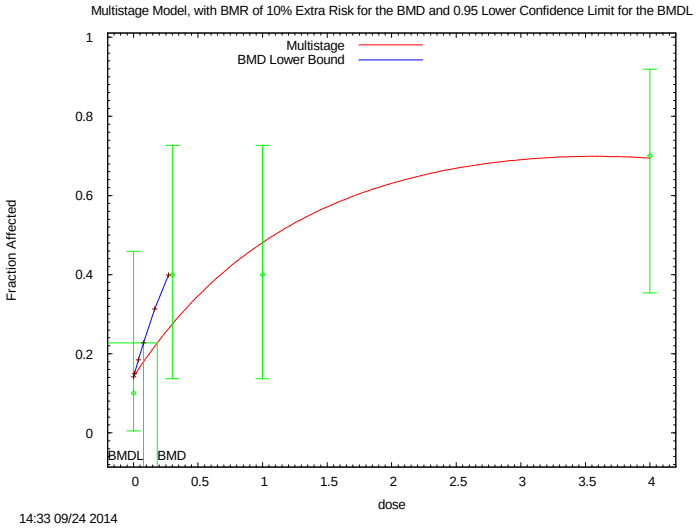
b.w.: body weight; na: not applicable.

Table H14: Study No 20: 0, 0.3, 1 and 4 mg Ni with incidences of clinically cutaneous reactions as of 1/10, 4/10,4/10 and 7/10

The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. When BMDL calculation failed the restricted models using BMDS-Software default values were calculated. The result with lowest $BMDL_{-10}$ of unrestricted models is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg Ni)	$BMDL_{10}$ (mg Ni)
Full model	na	4	22.82	–	–	–	–
Null (reduced) model	na	1	26.92	-	-	-	-
Probit	na	2	23.92	0.33	yes	0.70	0.45
LogProbit	none	3	23.07	0.48	yes	0.04	failed
	yes	2	24.06	0.29	yes	0.80	0.35
Logistic	na	2	23.93	0.33	yes	0.72	0.44
LogLogistic	none	3	23.06	0.48	yes	0.04	failed
	yes	2	24.06	0.29	yes	0.77	0.35
Quantal-Linear	na	2	23.67	0.43	yes	0.38	0.20
Multistage Cancer	na	2	23.67	0.43	yes	0.38	0.20
Multistage	**none**	**2**	**23.40**	**0.28**	**yes**	**0.18**	**0.08**
Weibull	none	3	23.00	0.52	yes	0.02	failed
	yes	2	23.67	0.43	yes	0.38	0.20
Gamma	none	3	23.00	0.55	yes	0.02	failed
	yes	2	23.67	0.43	yes	0.38	0.20

b.w.: body weight; if: invalid fit; failed: Benchmark dose computation failed. Lower limit includes zero; na: not applicable.



When combining the three studies, the increase of the incidences is not strictly monotone,

dose	0	0.3	0.4	2.5	1	3	4	5.6
incidence	4/10	5/10	5/10	6/20	9/9	7/10	6/6	

which prohibited a meaningful combined BMD analysis.

ABBREVIATIONS

% LC	Percentage of left-censored (data)
ERα	Estrogen receptor-α
$Ø_a$	Aerodynamic diameter
2-GEN	2-generation (study)
3- and 17-β-HSD	17-β-hydroxysteroid dehydrogenase
8-OH-dG	8-hydroxy-2'-deoxyguanosine
ACD	Acute contact dermatitis
AAS	Atomic absorption spectrometry
AdSV	Adsorptive stripping voltammetry
ADWG	Australian Drinking Water Guidelines
ALT	Alanine transferase
AP	Alkaline phosphatase
AT	Austria
ATSDR	Agency for Toxic Substances and Disease Registry
BE	Belgium
BG	Bulgaria
Bipea	Bureau Interprofessionnel d'Etudes Analytiques
BMD	Benchmark dose
BMDL	Benchmark Dose Lower-confidence Limit
$BMDL_{05}$	Lower 95 % confidence limit for a benchmark dose at 5 % extra risk
$BMDL_{10}$	Lower 95 % confidence limit for a benchmark response at 10 % extra risk
BMDU	Benchmark dose upper bound
BMR	Benchmark response
BP	Boiling point
BUN	Blood urea nitrogen
b.w.	Body weight
CAS	Chemical Abstracts Service
CHE	Chinese hamster embryo
CHO	Chinese hamster ovary
CI	Confidence interval
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
CY	Cyprus
CZ	Czech Republic
DE	Germany
DHS	California Department of Health Services
DK	Denmark
DNA	Deoxyribonucleic acid
DPCL	DNA-protein cross-link
DR	Dose-response
DRF	Dose range finding
d.w.	Dry weight
EC	European Commission
ED	Effective dose
EE	Estonia
EFSA	European Food Safety Authority
EFET	Hellenic Food Authority
EHEN	N-ethyl-N-hydroxyethylnitrosamine
EL	Greece
ES	Spain
EU	European Union
ETV	Electrothermal vaporization

ETV-ICP-MS	Electro-thermal vaporisation inductively coupled plasma mass spectrometry
EVM	Expert group on Vitamins and Minerals (UK)
F	Female
FAAS	Flame atomic absorption spectrometry
FAPAS	Food Analysis Performance Assessment Scheme
FI	Finland
FoodEx	EFSA Food classification and description system for exposure assessment
fpg	Formamidopyrimidine glycosylase
FR	France
FSA	Food Standard Agency (UK)
FSCJ	Food Safety Committee of Japan
GC	Gas chromatography
GD	Gestation day
GI	Gastrointestinal
GFAAS	Graphite furnace atomic absorption spectrometry
GLP	Good Laboratory Practice
GSH	Growth-Stimulating Hormone
HAT	Histone acetyltransferase
HBGV	Health-based guidance value
HD	High dose
HGP	Hypothalamic-pituitary-gonadal
HIF-1	Hypoxia-inducible transcription factor-1
HGPRT	Hypoxanthine-guanine phosphoribosyltransferase
HPLC	High performance liquid chromatography
HPG	Hypothalamic-pituitary-gonadal
HPLC	High performance liquid chromatography
HPLC-ECD	High performance liquid chromatography with electrochemical detection
HPRT	Hypoxanthine phosphoribosyltransferase
HU	Hungary
hyg	hygromycin (gene)
IARC	International Agency for Research on Cancer
IAEA	International Atomic Energy Agency (Austria)
IC	Ion chromatography
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IE	Ireland
IGGE	IGGE: Institute of Geophysical Exploration (China)
INCT	Institute of Nuclear Chemistry and Technology (Poland)
iNKT	Invariant natural killer cells T cells
i.p.	Intraperitoneal
IPCS	International Programme on Chemical Safety
IRMM	IRMM: Institute for Reference Materials and Measurements (Belgium)
IT	Italy
LB	Lower bound
LD	Low dose
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
LOQ	Limit of quantification
LPS	Lipopolysaccharide
LV	Latvia
M	Male
MD	Mid dose
MGP	Plant Waste Mixture
MHC	Major histocompatibility complex

MLs	Maximum levels
MOE	Margin of exposure
MOS	Margins of Safety
MP	Melting point
MRL	Minimal risk level
MS	Member State
MTD	Maximum tolerated dose
MW	Molecular weight
na	Not applicable
NA	Not assignable
Na_2 EDTA	Disodium ethylenediaminetetraacetic acid
Nd	Non detected
NER	Nucleotide excision repair
NHEXAS	National Human Exposure Assessment Study
ni	Not indicated
NIST	National Institute of Standards and Technology (USA)
NIES	National Institute for Environmental Studies (Japan)
NK	Natural Killer
NL	The Netherlands
NMIJ	National Metrology Institute of Japan (Japan)
NOAEL	No-observed-adverse-effect level
NR	Not reliable
NRC	National Research Council
NRCC	National Research Council of Canada (Canada)
NRK	Rat kidney cells
NTP	National Toxicology Programme
OEHHA	Office of Environmental Health Hazard Assessment
P5/25/50/75/95	5th/25th/50th/75th/95th percentile
PBK	Physiologically-based kinetic (model)
PKC	Protein kinase C
PND	Postnatal Day
PO	Poland
PT	Proficiency test
PTFE	Polytetrafluoroethylene
RBC	Red blood cell
REL	Reference exposure level
RfD	Reference dose
RHL	Recurrent herpes labialis
RIVM	National Institute for Public Health and the Environment
ROS	Reactive oxygen species
RP	Reference point
RvR	Rai-van-Ryzin (model)
RWC	Reasonable worst case
RWoR	Reliable without restrictions
RWR	Reliable with restrictions
s.c.	Subcutaneous
SCD	Systemic contact dermatitis
SCE	Sister chromatid exchange
SD	Sprague-Dawley (rats)
SE	Sweden
SGA	Small for gestational age
SI	Slovenia
SISE-EAUX	French Health and Environment Information System on Water database
SK	Slovakia
SLI	Springborn Laboratories Inc.

SNAS	Systemic nickel allergy syndrome
S-Ni	Serum nickel
SRL	Specific Release Limit
SRBC	Sheep red blood cells
SRM	Standard reference material
SSB	Single-strand break
TCEQ	Texas Commission on Environmental Quality
TDI	Tolerable daily intake
TDS	Total diet study
TERA	Toxicology Excellence for Risk Assessment
TI	Tolerable intake
TRH	Thyrothroin-releasing hormone
UB	Upper bound
UHT	Ultra High Treatment
UK	The United Kingdom
UL	Upper level
U-Ni	Urinary nickel
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UV	Ultraviolet
UVR	Ultraviolet radiation
WBC	White blood cells
WHO	World Health Organization
XPA	DNA repair protein complementing XP-A cells