



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### NICKEL GLUCONATE AND NICKEL SULPHATE

#### SUMMARY REPORT

1. Nickel gluconate and sulphate are indicated to be used in cases of nickel deficiency in horses, cattle, sheep, goats, pigs, chickens and rabbits. The recommended routes of administration are oral (as a liquid or a powder) or parenteral (intravenous, intraperitoneal, subcutaneous or intramuscular). For parenteral use the recommended doses, expressed in nickel equivalents, are 9 µg nickel/kg bw/day for single treatment of a young calf or in the range of about 1 to 6.5 µg nickel/kg bw/day during 4 days for repeated administration in all above mentioned species except chickens and rabbits. For oral use the range of doses is less than 1 up to about 22 µg nickel/kg bw/day administered for 4 to 5 days.
2. Nickel is an essential element. Nickel deficiency symptoms have been demonstrated using nickel-deficient diets in chickens, cows, goats, pigs, rats and sheep. Nickel was shown to improve the absorption of iron. It also interacts with calcium and zinc metabolism. It is a component of several enzyme systems. Urease activity in the rumen depends on nickel. Nickel is also needed for the action of some hydrogenases, dehydrogenases and transaminases, for instance malate dehydrogenase, isocitrate dehydrogenase and glutamic oxalate transaminase. Nickel may also affect the activity of α-amylases in the liver and pancreas and serve as a co-factor for the activation of calcineurin, a calmodulin-dependent phosphoprotein phosphatase.
3. The major routes of nickel intake for humans and animals are inhalation (occupational exposure, cigarette smoke, etc.) and ingestion (from food and drinking water) and, to a lesser extent, percutaneous absorption. Average total dietary intake of nickel is about 100 to 800 µg per day; about 1 to 10% of this amount is absorbed. In a US FDA study, 91% out of 234 foods contained nickel levels of less than 0.4 mg/kg and 66.2% contained less than 0.1 mg/kg. Seven foods had values above 1 mg/kg. In a Danish study the following mean nickel concentrations were found: milk, beef, pork, lamb: all 0.02 mg/kg (ranges up to 0.13 mg/kg for milk and 0.03 mg/kg for tissues); chicken: 0.11 mg/kg (range up to 0.24 mg/kg); liver and kidney (origin not specified): 0.11 mg/kg (range up to 0.94 mg/kg); fish: 0.04 mg/kg (range up to 0.3 mg/kg); eggs: 0.05 mg/kg (range up to 0.35 mg/kg). Mean concentrations in roots and vegetables were: 0.04 to 0.52 mg/kg (highest concentrations: in cabbage, peas and spinach: up to 3.99 mg/kg); fruits: 0.01 to 0.14 mg/kg (0.31 mg/kg in canned fruits: range up to 1.36 mg/kg); in wheat and rye flour, oatmeal and rice: 0.1 to 1.76 mg/kg. In general, food of animal origin has a relatively low nickel content. Rich sources of nickel are chocolate, nuts, dried beans and peas and grains.

4. The main excretory route of absorbed nickel in humans and animals is urine; biliary excretion is observed to a minor extent. In rats 3 to 6% of a  $^{63}$ labelled-nickel dose of 0.014 to 64 mg/kg bw  $\text{NiCl}_2$  administered by gavage was recovered in urine, while most radioactivity was found back in faeces. In mice 2% of 0.58 mg/kg  $^{63}$ nickel administered as  $\text{NiCl}_2$  in the diet was excreted in urine. Experiments with an *in situ* perfused intestinal loop preparation suggested that nickel absorption from the jejunum is a saturable process at concentrations greater than 20  $\mu\text{M}$ . The distribution and elimination of nickel, given parenterally to laboratory animals as  $^{63}\text{NiCl}_2$ , has been studied extensively. Most of the dose is rapidly excreted in urine (65 to 87% in 24 hours), the rest is eliminated much slower (76 to 90% in 5 days). Excretion in urine after single intravenous administration of  $^{63}\text{NiCl}_2$  in rats (82  $\mu\text{g}/\text{kg}$  bw) and rabbits (240  $\mu\text{g}/\text{kg}$  bw) followed a 2-compartment model with a rapid first order clearance phase during day 1 and 2 accounting for about 70% of the label and a much slower rate during days 3 to 7, reflecting possibly retention in tissues. After repeated oral exposure accumulation is possible, mainly in kidney, lung, liver, adrenals and cartilage.
5. Data from human volunteers after ingestion of a dose of 12, 18 or 50  $\mu\text{g}$  nickel/kg bw as nickel sulphate in drinking water or food fitted well in the two-compartment pharmacokinetic model described for rats and rabbits. Faecal elimination during 4 days after treatment was 76 % of the dose ingested in water *versus* 102% of the dose ingested in food. The mean elimination half-life was 28 hours (range 17 to 48 hours). Mean renal clearance was 5.8 (for food) to 8.3 (for water) ml/min/1.73  $\text{m}^2$ . In another experiment in humans, ingestion of soluble nickel in doses of 5 to 5.6 mg nickel (as 22 to 25 mg  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ ) per person resulted in increased serum concentrations within about 2.5 hours. Serum concentrations decreased to normal within 1 to 2 days. Ingestion of the same dose with meals did not increase serum concentrations. It has been estimated that in man, after inhalation, about 70% of nickel absorbed into the blood was excreted by the kidneys and that the remaining 30% was deposited in the tissues with a mean retention time of 200 days. A study comparing the kinetics of nickel (as sulphate) absorbed by volunteers from drinking water and food and excreted in urine, found that an average of 27% of the dose administered with water was absorbed as compared to an average of 0.7% of the same dose ingested with food.
6. In a large number of experiments, after parenteral (single or repeated) administration of soluble nickel salts to laboratory animals (rat, rabbit, mouse), in general shortly (up to 24 hours) after injection the highest concentrations were found in the kidneys, while one or more days after administration the highest concentrations were found in lung, heart or pancreas. Repeated oral administration resulted in some studies in a different distribution with higher concentrations in e.g. bones, liver, spleen, but in other studies again in the kidney (the reason for the distribution differences between studies could not be assessed, as only limited information was available). Shortly after intravenous injection of  $^{63}\text{NiCl}_2$  in mice in doses of 40 to 200 nickel/kg bw, radioactivity was found in kidneys, lungs, central nervous system, skin, cartilage and connective tissue. Three weeks later still radioactivity was found in kidney and lungs. Dietary administration of 1000 mg nickel/kg feed (as sulphate) to rats for 15 days resulted in increased nickel concentrations in kidney, testis, liver, spleen and myocardium. After dietary administration for 14 days of 10 and 20 mg nickel/kg to rats (as  $\text{NiCl}_2$ ) increased concentrations were found in kidney but not in liver and testis.
7. Total human body burdens of nickel in the range of 0.5 mg (for a 70 kg adult) to 5.7 mg (for 55 kg weighing Japanese adults) have been published. In older literature a body burden of 10 mg nickel has been indicated. Published ranges of mean reference values for nickel concentrations in human autopsy tissues were 7 to 44  $\mu\text{g}/\text{kg}$  for lung, 9 to 14  $\mu\text{g}/\text{kg}$  for kidney, 9 to 10  $\mu\text{g}/\text{kg}$  for liver, 6 to 8  $\mu\text{g}/\text{kg}$  for heart and 7  $\mu\text{g}/\text{kg}$  for spleen. In a study in tissues from Japanese humans the mean concentrations in the same tissues were 160  $\mu\text{g}/\text{kg}$  for lung, 100  $\mu\text{g}/\text{kg}$  for kidney, 80  $\mu\text{g}/\text{kg}$  for liver, lower than 300  $\mu\text{g}/\text{kg}$  for spleen and 100  $\mu\text{g}/\text{kg}$  for muscle.

8. Oral LD<sub>50</sub> values for nickel salts in rats and mice were 105 mg/kg bw and higher. Parenteral LD<sub>50</sub> values of nickel salts were much lower: between about 20 and 100 mg/kg bw.
9. Overviews of several repeated dose toxicity studies with little details on experimental design and results were available. Toxicity studies with a duration of 8 weeks in rats (doses 0, 250, 500 and 1000 mg/kg feed as nickel carbonate), calves (0, 62.5, 250 and 1000 mg/kg feed as nickel carbonate) and 24 weeks in monkeys (0, 250, 500 and 1000 mg/kg feed as nickel carbonate) showed no significant effect on body weight in rats and monkeys and on haematological parameters in monkeys but caused signs of toxicity (reduced feed intake, body growth and organ weights and kidney lesions) at 250 and 1000 mg/kg feed in calves. Toxicity studies with administration of nickel chloride in the diet or drinking water of rats, with a duration of 4 to 40 weeks revealed effects on body weight and blood biochemistry at doses of 2.5 mg/l drinking water during 4 weeks and higher and at 20 mg/kg feed. In a 7-month nickel sulphate gavage study in rats with doses of 0, 0.0005, 0.005, 0.05, 0.5 and 5 mg/kg bw/day of nickel, significant effects were only found at the highest dose level (lack of weight gain, histopathological changes in the intestines: extensive proliferation of lymphoid cells and histiocytes and micronecrosis). In a 6-week toxicity study with nickel acetate in the diet at doses of 10, 50 or 100 mg/kg bw/day, the mid and high dose animals showed decreased body weight and haematological effects. In a 120-day gavage study, 25 mg/kg bw/day of nickel sulphate caused degenerative cellular changes in liver and kidney, decreased testis weight and histological changes in the testis. Rats fed a diet containing nickel acetate at doses of 16 to 160 mg nickel/kg bw/day for 10 to 190 days showed high mortality, and various histopathologic effects. As incomplete information about these and other studies was available, no NOEL could be based on the available data.
10. In a 2-year toxicity study rats and beagle dogs received 0, 100, 1000 and 2500 mg nickel/kg feed as nickel sulphate hexahydrate (no actual doses reported, estimated doses: 0, 5, 50, 125 mg/kg bw/day for rats and 0, 2.5, 25 and 62.5 mg/kg bw/day for dogs). In rats (both sexes) a dose related decreased body weight gain was found at the mid and high dose levels. After 2 years, relative heart weight was increased and relative liver weight was decreased in female rats at the mid and high dose levels. At 2 years the tissue concentrations in bone, liver, kidney and fat were the following: control females: 530, 94, 140, and 510 µg/kg, respectively; control males: lower than 96, 55, lower than 140 and lower than 55 µg/kg, respectively; high dose females: 820, 640, 3400 and 1000 µg/kg, respectively; high dose males: 640, 680, 4900 and 1400 µg/kg, respectively. In the dog study a decrease of body weight, decreased hematocrit and hemoglobin values, high urine volumes in some dogs and increased relative weight of liver and kidney were found in the high dose group. In addition animals of this group showed histopathological changes in the lungs (all animals) and granulocytic hyperplasia of the bone marrow (2 out of 6 dogs) The highest tissue concentration was found in kidney of the high dose group: 4000 to 7000 µg/kg. Approximately 1 to 3% of the ingested dose was excreted in urine. NOELs for nickel in the rat and the dog study were 5 and 25 mg/kg bw/day, respectively.
11. Calves fed 1000 mg/kg feed of nickel (no information was available about the administered compound) showed lowered retention of total nitrogen, calcium and phosphorous, decreased food intake and growth. The growth rate was reduced in chickens fed 900 to 1300 mg/kg feed of nickel (as the acetate or sulphate).

12. Incomplete information on teratogenicity of nickel was available. Conflicting results on teratogenic effects of NiCl<sub>2</sub> in chicks have been found (cardiac anomalies, exencephaly and distorted skeletal development in some studies and no anomalies in other studies). In two intraperitoneal teratogenicity studies in rats (doses 1 to 4 mg/kg bw as the chloride) and mice (doses 1.2 to 6.9 mg/kg bw nickel as the chloride) dose related foetotoxicity and serious malformations (acephaly, exencephaly, cleft palate, club feet and several other malformations) were found. In two intramuscular studies in rats with doses in the same range, no teratogenic effects were found. As incomplete information about these and other studies was available, no overall NOEL for teratogenicity could be based on the available data.
13. A three generation reproduction study was carried out in rats with doses of 0, 250, 500 and 1000 mg nickel/kg feed as nickel sulphate hexahydrate (no actual doses reported, estimated doses: 0, 12.5, 25, 50 mg/kg bw/day). Slight decreases of parental body weights were found at the high dose level. The number of pups born dead was only increased in the first generation at all dose levels. The number of pups alive at weaning showed a dose related decrease, with only a clear adverse effect at the highest dose level. Body weight of weaned pups was decreased at the high dose level. Gross observations revealed no teratogenic effects. No histopathological effects were found in F3b pups at weaning. No clear NOEL could be established. In a number of other studies in rats and other laboratory animals (routes: inhalation, oral, subcutaneous) testicular toxicity was found.

As the available information about these and other studies was very incomplete, no overall NOEL for reproduction could be based on the available data.

14. A large number of published mutagenicity studies on nickel and nickel salts (nickel chloride, nickel sulphate, nickel nitrate and nickel acetate) was summarised and assessed by the International Agency for Research on Cancer (IARC) in 1990. Several of these studies revealed positive results, in particular in *in vitro* mutagenicity tests in eukaryote test systems. Of the available *in vivo* mutagenicity tests some were negative and others positive, but in all these tests the intraperitoneal route was used. There were no tests with oral administration. In addition elevated chromosomal aberrations were found in blood cells from workers in nickel industries exposed to nickel salts (chloride and sulphate) or other nickel compounds by inhalation. The International Agency for Research on Cancer concluded that soluble nickel compounds were generally active in the *in vitro* assays of human and animal cells in which they were tested. Based on this information it was concluded that there is evidence showing that the nickel salts used in veterinary medicine may be mutagenic.
15. A number of published carcinogenicity studies on nickel and nickel compounds was summarised and assessed by the International Agency for Research on Cancer in 1990 and the World Health Organization (WHO). Several of these studies revealed positive results, in particular tumours at the place of administration. The examined nickel salts, nickel chloride, sulphate and acetate induced malignant tumours in the peritoneal cavity after intraperitoneal injection in rats. Intramuscular injection in rats of nickel sulphate gave no increase of local tumours. In mice, intraperitoneal injection of nickel acetate induced malignant tumours in the peritoneal cavity and the lungs. Epidemiological studies showed increased frequencies of lung and nasal cancer in nickel industry workers. The International Agency for Research on Cancer concluded that there is sufficient evidence in humans for the carcinogenicity of nickel sulphate and that there is limited evidence in experimental animals for the carcinogenicity of nickel salts. The overall conclusion, based on the evaluated information and the underlying concept that nickel compounds can generate nickel ions at critical sites in their target cells, was that nickel compounds are carcinogenic to humans. Although no carcinogenicity was found in oral long term studies in laboratory animals reviewed by the World Health Organization, the available studies were considered insufficient. From the available evidence, the Committee concluded that, considering the likely carcinogenicity of nickel ions, it cannot be excluded that increased exposure to nickel residues in edible products originating from treated animals may represent an increased risk for consumers. Therefore, significantly increased exposure of consumers to nickel in the

daily diet was not considered acceptable.

16. Literature data reviewed by the World Health Organization indicate that nickel (at least after parenteral administration or instillation in the lungs) may exert immunodepressive effects (reduced host resistance to viral and bacterial infections, suppression of phagocytic capacity of macrophages, suppression of natural killer cells). No data were available to establish an oral NOEL.
17. Oral exposure to nickel has been shown to elicit hypersensitivity symptoms in sensitized persons. Nickel sulphate in a single dose of 0.6 mg or higher or a dose of 0.4 mg or higher on two repeated days (doses expressed as elementary nickel) can elicit positive dermal responses in nickel-sensitive patients. A diet with low nickel content may diminish the activity of hand eczema in some nickel-sensitive patients and a flare of hand eczema has been seen in patients who abandoned such a diet. A diet naturally containing about 500 µg nickel (chocolate cake, soya bean stew and oatmeal) resulted in an exacerbation of vesicular hand eczema starting 7 days after a 4 day period of ingestion. According to the International Agency for Research on Cancer nickel ions are considered to be exclusively responsible for the immunological effects of nickel. These data suggest that normal dietary exposure may already elicit hypersensitivity reactions in sensitized persons and that any significant increase in the nickel content of the daily diet as a consequence as the use of nickel as veterinary medicine may increase the risk of occurrence of hypersensitivity signs in sensitized consumers.
18. The World Health Organisation established a Tolerable Daily Intake for nickel in drinking water of 5 µg/kg bw/day and a maximum acceptable concentration in drinking water of 20 µg/l, based on a NOAEL of 5 mg/kg bw/day for changes in organ weights in a 2-year rat study and a safety factor of 1000. The safety factor contained an extra factor of 10 because insufficient oral carcinogenicity data were available and because of the higher bioavailability of nickel administered in an empty stomach as compared to nickel administered in the presence of food.
19. The Committee concluded that insufficient information was available to establish a toxicological NOEL and ADI. The NOEL of 5 mg/kg bw/day established by the World Health Organisation was not taken over, because there was evidence that toxic effects were found in at least one study at a dose level of 5 mg/kg bw/day. However, because the study was not available for assessment no final conclusion regarding an overall NOEL was possible. In addition, there is evidence that nickel ions are genotoxic carcinogens and that increased exposure to nickel ions in the daily diet may induce hypersensitivity reactions in sensitised persons.
20. Calves receiving 0, 3, 12 and 14 mg/kg bw/day of nickel (as the carbonate) for 8 weeks showed a dose-dependent increase of urinary nickel concentrations. The nickel concentration in liver did not increase significantly at any dose. The concentration in kidney increased only at the highest dose level (from 2.08 µg/kg dry matter in the controls to 22.8 µg/kg dry matter in the high dose group). Nickel concentrations in a number of other organs increased slightly in a dose related way (highest concentration: 5.88 µg/kg dry matter in lung), but only significantly at the highest dose. Concentrations in fat and muscle were not measured. The fact that in spite of the small difference in intake between the mid and the high dose group only significant increases in tissue levels were found in the high dose group, suggests that a homeostatic mechanism exists for nickel content.

Lambs received for 97 days 65 or 5065 mg/kg feed of nickel as NiCl<sub>2</sub>. On day 94 a single oral dose of 4.3 µg <sup>63</sup>NiCl<sub>2</sub>/kg metabolic body weight was given. The highest percentage of this radioactive dose was recovered in kidney (13% in the low dose group and 32% in the high dose group), the percentage of the dose recovered from lung, spleen, heart, testis liver and brain was 2% or less in both groups (concentrations were not reported). Based on these data the oral doses recommended for therapeutic administration (maximally 2.6 mg/animal day of nickel) would not be expected to result in increased concentrations in kidney and liver. Since from the general distribution of nickel it is known that these organs generally contain higher concentrations than muscle and fat, no significant increase

would be expected in these tissues either.

21. Due to a lack of available data, residue concentrations after the administration of nickel salts in milk, eggs after oral or parenteral administration and edible tissues and injection site after parenteral administration could not be assessed. However, the maximum estimated parenteral dose (780 µg/animal/day) is in the order of magnitude of the maximum already existing exposure through the daily human diet (100 to 800 µg/day). After absorption of the parenteral dose, only a limited part of this dose could be expected to be present in the amounts of edible tissues maximally assumed to be consumed as a part of the daily diet. The maximum oral dose is up to about three times as high, but taking into account the low oral bioavailability of nickel salts (1 to 10% from food, but up to about 30% from drinking water), only a limited proportion of this dose could be expected to produce residues in edible tissues. For these reasons, nickel residues in standard edible products (tissues, milk and eggs) were not expected to increase significantly daily dietary intake of nickel by consumers.

### Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II to Council Regulation (EEC) No 2377/90 and in particular that:

- nickel is a normal component of the daily human diet,
- nickel is seldomly used in veterinary medicine,
- animals are unlikely to be sent for slaughter immediately after treatment,
- nickel has a low oral bioavailability and a high percentage of the absorbed part of the dose is excreted within 24 hours,
- the use of nickel gluconate and sulphate for the treatment of nickel deficiency diseases in food-producing animals is not expected to increase substantially the total dietary exposure of the consumer to nickel;

the Committee considers that there is no need to establish an MRL for nickel gluconate and nickel sulphate and recommends their inclusion in Annex II to Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Nickel gluconate	All food producing species	
Nickel sulphate	All food producing species	