Brief profiles on systemic toxicological effects of common counter-ions in metal substances:

8. Nitrate

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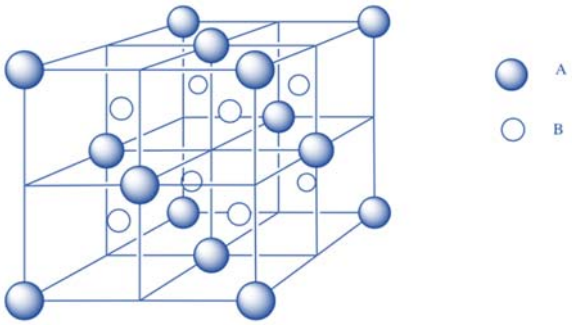

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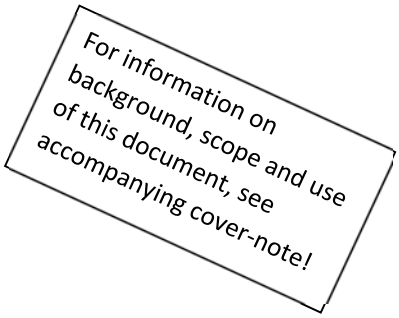
Nitrate: Brief profile on systemic toxicological effects



1. Identification

Name: nitrate (anion)
Chemical formula: NO_3^-
Molecular mass: 62.01 g/mol

2. Introductory remarks

As an ion, nitrate is not a substance under REACH, so that toxicological information is not available on the ion on its own. Nitrate is a common anion in many salts of cationic metals. The aim of this summary on the toxicity of nitrate is to assist the assessor of health hazards of nitrate containing substances, in determining whether the nitrate moiety contributes to the overall toxicity of the substance. This document does not discuss primary literature or studies in great detail. Instead, reference is made to most recently published authoritative reviews, such as from the European Food Safety Authority. If no other citation is given, statements in this document have been either copied or adopted from EFSA (2017).

3. Natural occurrence, physiological function and dietary intake

Sodium (E 251) and potassium (E 252) nitrates are authorised as food additives in the European Union (EU) according to Annex II to Regulation (EC) No 1333/2008 on food additives and they were previously evaluated by the EU Scientific Committee for Food (SCF), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA).

4. Toxicokinetics

There are ample data available on the toxicokinetics in humans (EFSA, 2017), so that data in animals are not referred to here.

In humans, dietary nitrate is rapidly and extensively absorbed through the gastrointestinal tract (Iijima et al., 2002). Nitrate is found in high concentrations in the saliva due to its secretion by salivary glands, and bacteria can convert the secreted nitrate into nitrite. In the gastrointestinal tract, nitrite is absorbed and thereafter enters the general circulation where it can be oxidised by haemoglobin to form nitrate and methaemoglobin.

Nitrate is metabolised to a minor extent. Its biotransformation comprises nitrate reduction, nitrite formation, nitrite reoxidation to nitrate, and formation of methaemoglobin or NO, in a dynamic equilibrium (Gladwin et al., 2005; Lundberg et al., 2008). Due to the very low gastric pH, very little further reduction of nitrate to nitrite occurs in man (Mirvish, 1975).

Between 70% (Wagner et al., 1983) and 100% (Pannala et al., 2003) of a nitrate dose is excreted by the kidneys.

The volume of distribution was calculated to be between 0.24 and 0.44 L/kg bw (mean 0.32 L/kg bw) (Lambers et al., 2000). Since the volume of distribution is smaller than the body water and higher than the blood volume, this indicates that nitrate is readily distributed throughout the body. Nitrate is also found in the milk of lactating women where the concentration is similar to that in the plasma (Green et al., 1982). Nitrate is mainly converted to nitrite in a specific mechanism.

In summary, in humans, nitrate is systemically available to 100%. Nitrate is secreted into saliva (20–25% of the dose) and converted to nitrite by bacteria in the mouth (5–36%). Nitrite is absorbed and re-metabolised to nitrate. Small amounts of nitrite are metabolised to NO and reactive oxygen species and 0.02% of a dose of nitrite is found in the urine. Most of nitrate is excreted into the urine, with the amounts varying between 50% and 100%.

5. Toxicological effects

It is not practically possible to address standard toxicological data requirements for the nitrate anion on its own. The information in the subchapters below is therefore primarily based on substances such as sodium or potassium nitrate. These two substances are reasonably well researched due to their use as food additives (E251/252).

5.1. Acute toxicity

Acute oral values (LD_{50}) for sodium nitrate were reported to range between 2,480 and 6,250 mg sodium nitrate/kg_{bw} for mice, and between 4,860 and 9,000 mg sodium nitrate/kg_{bw} for rats. For rabbits, a LD_{50} value of 1,600 mg/kg bw has been reported (Corré & Breimer, 1979; RIVM, 1989).

5.2. Sensitisation

Data on sensitising properties of nitrates are scarce in the public domain: EFSA (2017) did not address sensitising properties at all in their review on sodium and potassium nitrates as food additives. A recent ATSDR Toxicological Profile for Nitrate and Nitrite (2017) concludes that no information was located regarding immunological and lymphoreticular effects in humans or animals exposed to nitrate or nitrite via the inhalation, dermal or oral route. OECD also did not report reliable data on sensitisation when agreeing on a screening information dataset on the category of nitrates (OECD, 2007) nor for sodium nitrite (OECD, 2005).

Available data in the public domain in most cases refer to nitrate salts which are either (i) irritating or corrosive and therefore cannot be tested in standards tests (e.g. $AgNO_3$), or (ii) or represent substances in which the metal cation itself elicits skin sensitising effects (e.g. $Ni(NO_3)_2$), so that conclusions on the nitrate moiety itself cannot be drawn.

However, it is noted that the ECHA Brief Profile on sodium nitrate states on skin sensitisation: “No adverse effect observed (not sensitising)”, which is based on one key study (ECHA, 2019).

Authors comment: The study in question is described in the disseminated dossier as an OECD TG 429 LNAA with NaNO3, performed in the year 2010 (also referred to in the dossier on potassium nitrate, by way of read-across). When citing this information, the user should be aware that data access from the data holder needs to be ensured.

5.3. Repeated dose toxicity

Short-term and subchronic toxicity studies in rats showed, overall, that nitrate intake of up to 5% in the diet (equivalent to 4,500 mg sodium nitrate/kg_{bw} per day) did not result in adverse effects in rats. At higher dose levels, animals showed signs of methaemoglobinaemia leading to the death of the animals (EFSA, 2017).

The current acceptable daily intakes (ADIs) for sodium and potassium nitrate (expressed as nitrate ion) established by the SCF (1997) and JECFA (2002) are 0 - 3.7 mg nitrate/kg body weight (bw) per day, as reconfirmed by EFSA (2017).

5.4. Mutagenicity/genotoxicity

In vitro studies on sodium and potassium nitrate in bacteria and mammalian cells did not provide evidence of any genotoxic potential. In mammals, no reliable indication of genotoxicity was obtained in mice and rats exposed to nitrate by the oral route, both in somatic and in germ cells. Although the database is limited, the EFSA Panel concluded that the available experimental data indicated that nitrate salts do not raise concern for genotoxicity (EFSA, 2017).

5.5. Reproductive toxicity

In female guinea-pigs exposed via drinking water to potassium nitrate at concentrations up to 30,000 mg/L (equal to 1130 mg/kg bw/d), the mating behaviour was highly impaired only at the highest concentration of 30,000 mg/L, whereas the fertility was not reduced at lower doses. Weight gain and food and water intake were normal at all concentrations. No macroscopic or microscopic alterations were observed in the reproductive organs (Sleight & Atallah, 1968; Speijers et al., 1989; WHO, 1996).

In a two-generation rabbit study, sodium nitrate at dose levels of 0, 8, 250 or 500 mg/L in drinking water had no effect on the number of pregnancies, litter size or pup weights. (Kammerer, 1993; Kammerer & Siliart, 1993; Speijers et al., 1989; WHO, 1996).

In an OECD TG 422 reproductive/developmental toxicity screening study conducted under GLP, rats were exposed to up to 1,500 mg/kg bw/d of potassium nitrate by gavage The NOAEL for reproduction and developmental toxicity was 1,500 mg/kg bw/day based on the absence of adverse effects (OECD SIDS, 2007).

Two independent developmental studies in rats are referred to by JECFA (2003): sodium nitrate was administered to Wistar rats via gavage at doses up to 250 mg/kg bw/d. No adverse effects were observed in dams or fetuses (FDRL, 1972a). In a similar study in Wistar rats with potassium nitrate, dosing via gavage at up to 180 mg/kg bw/d did not elicit adverse effects either in dams or fetuses (FDRL, 1972b).

Corresponding studies in rabbit are likewise referred to by JECFA (2003): Dutch-belted rabbits were dosed via gavage at up to either 250 mg/kg bw/d with sodium nitrate or up to 206 mg/kg bw/d with potassium nitrate. In both studies, incidences of corpora lutea, implantations, live and dead fetuses, and resorptions in dams were within the normal range and there were no increased incidences of external, visceral and skeletal abnormalities and also fetal weights were not affected. However, due to the low number of dams with live litters, the JECFA Panel considered these studies as not relevant for risk assessment (FDRL, 1972a and 1972b).

In a 35d drinking water study in mice with potassium nitrate at concentrations corresponding to doses of up to 156.6 mg/kg bw/d, a decrease in sperm count and motility, and an increase in total percentage on sperm abnormalities and histopathological changes in the testes of the males of the highest dose group (NOAEL 122 mg/kg bw/d) were observed. However, definitive conclusions are not possible based on this study due to the short study duration and low number of animals tested (Pant and Srivastava, 2002).

Overall, based on the available data, substances such as sodium and potassium nitrate are not considered reproductive or developmental toxicants (OECD SIDS, 2017).

5.6. Carcinogenicity

Chronic toxicity and carcinogenicity studies with sodium and potassium nitrate are available. In studies with mice, sodium nitrate did not show any difference in tumour incidences compared to controls. Four non-standard studies in rats and pigs assessed haematological parameters or effects on thyroid and thyroid-related hormones (Boink et al., 1995; JECFA, 1996; Zaki et al., 2004; Mukhopadhyay et al., 2005; Azeez et al., 2011).

Overall, the EFSA Panel (2017) considered that nitrate did not affect adrenal and thyroid glands function in animals, and it was not carcinogenic in animal studies. The EFSA Panel (2017) also reviewed human epidemiological studies, and concluded that there was no evidence for a positive association between ingested nitrate and, for example, oesophageal, gastric, colorectal or pancreatic cancer.

6. Discussion and conclusion

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provided a scientific opinion on the safety of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives in 2017. In this review, the acceptable daily intake (ADIs) for nitrate of 0 - 3.7 mg/kg body weight (bw) per day as previously established by SCF (1997) and JECFA (2002) was confirmed. The exposure to nitrate solely from its use as a food additive was estimated to be less than 5% of the overall exposure to nitrate in food based on a refined estimated exposure scenario. This exposure did not exceed the current ADI (SCF, 1997).

Given that it is known that nitrate in the body can be converted to nitrite with subsequent potential formation of methaemoglobin, the EFSA Panel also considered the derivation of an ADI for nitrate based on this. However, there were large variations in the data on the nitrate-to-nitrite conversion in the saliva in humans. Therefore, the Panel considered that it was not possible to derive a single value of the ADI from the available data. However, it was nevertheless concluded that the methaemoglobin levels produced due to nitrite obtained from this conversion would not be clinically significant.

The available data did not indicate any genotoxic potential for sodium or potassium nitrate. The carcinogenicity studies in mice and rats were negative, and there is no indication that substances such as sodium and potassium nitrate are reproductive or developmental toxicants.

Nitrate “in a nutshell”:

Key / leading adverse systemic effect on human health:	conversion of nitrate to nitrite with subsequent formation of methaemoglobin
Relevant (CLP) Hazard Classification(s) for systemic effects:	none
Numerical toxicological descriptor:	acceptable daily intakes (ADIs) for sodium and potassium nitrate (expressed as nitrate ion): 0 - 3.7 mg/kg established by SCF (1997) and JECFA (2002), confirmed by EFSA (2017)

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