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

5. Ammonium

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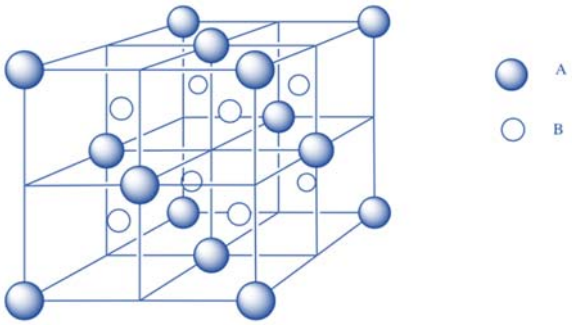

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Ammonium: Brief profile on systemic toxicological effects

1. Identification

Name: ammonium (cation)
Chemical formula: NH_4^+
Molecular mass: 18.04 g/mol

2. Introductory remarks

As an ion, ammonium is not a substance under REACH, so that toxicological information is not available on the ion on its own. Ammonium is a common cation in salts e.g. of halogens or (oxo)anionic metals. The aim of this summary on the systemic toxicity of ammonium is to assist the assessor of health hazards of ammonium containing substances, in determining whether the ammonium moiety contributes to the overall toxicity of the substance.

This document does not discuss primary literature or studies detail. Instead, reference is made to most recently published authoritative reviews on the topic, 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 (2011), which is the most recent opinion by an EFSA panel including a thorough assessment of the toxicity of ammonium substances. The majority of the toxicological data presented here refers to the substance ammonium chloride, a widely used, readily soluble salt, in which the "toxicity depends on ammonia [and not the chloride] which enters the living organism and thence the cell" (OECD, 2003).

3. Natural occurrence and dietary intake

Ammonia and ammonium ions are present in aqueous environments as well as under physiological circumstances at different ratios depending on the pH. Ammonia is produced endogenously in all mammalian species. In the gut, ammonia is produced by bacterial degradation of nucleic and amino acids from ingested food. The estimated production of ammonia in the human intestine may range from 10 mg/day in the duodenum to 3 g/day in the colon.

Ammonium salts have been ingested for a long time by humans. For example, ammonium chloride can be added directly to human food as it is authorised in the EU as a food flavouring, together with ammonia, ammonium sulfite and ammonium hydrogen sulfite. NH_4Cl is considered in the United States as "Generally Recognized As Safe" (GRAS). NH_4Cl is approved as a drug in several countries for electrolyte replenishment or expectorants and as food additive (fermentation and blowing agent) without usage restrictions in Japan. The toxicity of ammonium chloride depends on ammonia which enters the living organism and thence the cell (OECD, 2003).

When EFSA (2011) assessed four ammonium substances, information on actual intakes was scarce and two different methods were used to derive indicative estimates of intakes. By use of the "Maximised Survey-derived Daily Intake" (MSDI) approach and the "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach, the resulting intake estimates were considerably below or above the thresholds of concern, respectively. The EFSA Panel had reservations about the data on use and use levels provided and the intake estimates obtained and was unable to derive realistic intake estimates.

4. Toxicokinetics

Ammonia is readily absorbed from the gastrointestinal tract, after which it enters the portal circulation and is transformed to urea in the liver via the urea cycle and is subsequently excreted as urea via the kidneys (EFSA, 2011). Ammonium chloride is easily absorbed. EFSA considers that ammonium salts or ammonia may be predicted to be metabolised to innocuous products in the human body at estimated levels of exposure as flavouring substances, according to the MSDI approach.

5. Toxicological effects

Ammonia/ammonium is produced endogenously in all mammalian species and not considered as "toxic" per se. However, adverse health consequences could be possible at excessive doses. The majority of the toxicological data presented here refers to the substance ammonium chloride, a widely used readily soluble salt, in which the "toxicity depends on ammonia [and not the chloride] which enters the living organism and thence the cell" (OECD, 2003).

5.1. Acute toxicity

EFSA cites an acute oral LD_{50} of 1650 mg/kg in rats for ammonium chloride. This is in agreement with the harmonised EU hazard classification of NH_4Cl as Acute Tox 4, H302: Harmful if swallowed (CLP Regulation Index No. 017-014-00-8). The LD_{50} of 1650 mg NH_4Cl/kg corresponds to a LD_{50} of 556 mg NH_4/kg.

5.2. Sensitisation

OECD (2003) reports that a guinea pig maximization test conducted according to GLP was negative for NH_4Cl, showing that the substance, and thus also the ammonium moiety, does not have sensitising potential.

5.3. Repeated dose toxicity

The OECD SIDS for ammonium chloride (OECD, 2003) identified a 70-day repeated dose toxicity study by Arnold et al. (1997) as the most reliable. This study resulted in an unbounded NOAEL (highest dose tested) of 684 mg NH_4Cl/kg_{bw}/d. Later, Lina and Kuijpers (2004) studied the toxicity and carcinogenicity of acidogenic or alkalogenic diets in rats, using ammonium chloride as one of the test substances. In the repeated dose toxicity part of the study, which was similar to OECD TG 408, two dose groups were fed with 2.1% and 4 % NH_4Cl in the diet (nominal) for 13-weeks, resulting in nominal doses of 1,696 and 3,373 mg/kg_{bw}/d. Based on a body weight reduction of up to 20% seen in both sexes of the high dose group, the sub-chronic NOAEL was 1,696 mg NH_4Cl/kg_{bw}/day. See below for a summary of the subsequent chronic toxicity and carcinogenicity study by the same authors.

5.4. Mutagenicity/genotoxicity

OECD (2003) summarised the available studies on ammonium chloride as follows: "A reverse mutation study in bacteria [OECD TG 471] gave negative results. An in vitro chromosomal aberration test with Chinese hamster lung cells (CHL/IU) without metabolic activation was positive. This result is ascribable to the acidity of this substance. An in vivo micronucleus assay up to the maximum tolerance dose was negative. Based on the weight of evidence, this substance is considered to be non-genotoxic."

5.5. Reproductive toxicity

No information was located regarding the reproductive or developmental effects of ammonia or ammonium compounds in *humans* (ATSDR, 2004).

Rats pups exposed to NH_4^+ in utero and during lactation (dams received 4,293 mg NH_4^+/kg_{bw}/d in the diet from gestational day 1 through lactation day 21), which then received a normal diet, had a statistically

significant reduction in body weight gain (Miñana et al. 1995). No information was provided in the study regarding the health of the dams, including no information on body weight or food consumption, rendering a full assessment impossible. EFSA (2011) considers that it is likely that the high ammonium dietary concentration made the dams hyperammonemic and that the effect (reduced body weight gain in off-spring) at such high maternal dose levels (>4000 mg/kg_{bw}/d) was most likely the result of maternal toxicity.
OECD (2003) summarised a further teratogenicity study by Goldman and Yakovac (1964) as follows: 10 females Sprague-Dawley rats were administered by gavage once a day with 8.9 mg NH_4Cl/kg_{bw}/d) on days 7 to 10 of gestation. The fetuses were obtained by Cesarean section and examined on day 20 of gestation. Maternal effect was supposedly acidosis, no teratogenic toxicity was found. The effects of this substance on fetal growth noted were considered a consequence of maternal acidosis. No fetus with malformations was observed in this substance-treated group. This substance [NH_4Cl] has no teratogenicity at this dose level.
In conclusion, and considering the fact that ammonium/ammonia are produced endogenously in all mammalian species including humans in considerable quantities during digestive processes, it can be concluded that ammonium does not adversely affect reproductive functions or would be teratogenic.

5.6. Carcinogenicity

Lina and Kuijpers (2004) studied the toxicity and carcinogenicity of acidogenic or alkalogenic diets in rats, using ammonium chloride as one of the test substances. In the carcinogenicity part of the study, which was similar to OECD TG 451, two dose groups were fed for 30 months with 1% and 2.1% NH_4Cl in the diet (nominal). The doses were selected based on the preceding 13-week studies which showed marked body weight reduction at 4% NH_4Cl in the diet (see above). Actual doses received in the carcinogenicity study are reported as 503 and 1,105 mg/kg_{bw}/d. Treatment with NH_4Cl did neither affect type, incidence and multiplicity of tumours, nor time of tumour appearance and the ratio benign-malignant tumours. The rats showed a remarkable adaptive capacity to the life-long exposure to NH_4Cl and no adverse effects were noted in either dose group. Thus, a chronic NOAEL can be set at 1,105 mg NH_4Cl/kg_{bw}/d, which corresponds to 372 mg NH_4/kg_{bw}/d.

6. Discussion and conclusion

Based on information largely available for the readily soluble salt NH_4Cl, the ammonium moiety can be regarded as non-toxic up to doses of 1,105 mg NH_4Cl /kg/day, which was an unbounded NOAEL established in a 30-month toxicity and carcinogenicity study (Lina and Kuipers, 2004). Significant effects on body weight were only reported an even higher dose of 3,373 mg NH_4Cl /kg_{bw}/d in a 13-week study by the same authors, which has the sub-chronic NOAEL set at 1,696 mg NH_4Cl/kg_{bw}/d.
Thus, the chronic NOAEL of 1,105 m mg NH_4Cl /kg_{bw}/d, which corresponds to 372 mg NH_4/kg_{bw}/d can be taken as the basis for the assessment of the toxicity of the ammonium ion. Note that this an animal NOAEL and that the discussion of assessment factors which are required to derive DNELs for humans is not within the scope of this document.
Regarding the chloride moiety in NH_4Cl, as indicated above, OECD (2003) considered that the ammonium moiety is the driver for any toxicity of this substance. EFSA (2019) has recently reviewed information on possible adverse effects by chloride. The EFSA Panel noted the close relationship between sodium and chloride balances in the body, and concluded that there is insufficient data to assess toxicological effects (and set an upper intake level) for chloride on its own. Hence, the EFSA Panel considered that reference values for chloride can be set at values equimolar to the reference values for sodium (see “counter ion document on chloride”).

Ammonium "in a nutshell":

Key / leading adverse systemic effect on human health:	Effects on body weight in rats at very high doses (> 4000 mg $NH_4Cl/kg_{bw}/d$ or >1300 mg $NH_4/kg_{bw}/d$), likely due to hyperammonemia and/or acidosis.
Relevant (CLP) Hazard Classification(s) for systemic effects:	none
Numerical toxicological descriptor:	Chronic rat NOAEL: 372 mg NH_4/kg/day

References

ATSDR (2004): Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ammonia (TP 126).

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Miñana MD, Marcaida G, Grisolia S, et al. 1995. Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. J Neuropathol Exp Neurol 54(5):644-650.

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