Nitrilotriacetic acid and its sodium salts

MAK Value Documentation, supplement – Translation of the German version from 2020

A. Hartwig[1,*]   MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the Carcinogen Category, the maximum concentration at the workplace (MAK value) and the Pregnancy Risk Group of nitrilotriacetic acid [139-13-9] and its sodium salts [18994-66-6, 15467-20-6, 23255-03-0, 5064-31-3, 18662-53-8]. Available publications and unpublished study reports are described in detail. Nitrilotriacetic acid and its sodium salts cause an increased incidence of carcinomas of the kidneys and urinary tract in rats and mice. Underlying mechanisms are cytotoxicity and, at higher doses, potential indirect genotoxicity. These processes are due to the complex-forming properties of nitrilotriacetic acid. As their thresholds have been examined in detail, nitrilotriacetic acid and its sodium salts are classified in Carcinogen Category 4. A NAEL (no adverse effect level) of 2.3 mg/kg body weight and day for hyperplasia of the urinary bladder epithelium, the most sensitive end point, was estimated for nitrilotriacetic acid from a 2-year study in rats. Based on this NAEL, the MAK value for nitrilotriacetic acid and its sodium salts is set at 2 mg/m^3 I as acid. Since the critical effect of nitrilotriacetic acid and its sodium salts is systemic, they are assigned to Peak Limitation Category II. As the half-life of nitrilotriacetic acid in humans is approximately 4 hours, an excursion factor of 4 is set. There is an adequate margin between the NOAEL for developmental toxicity scaled to a concentration at the workplace and the MAK value. Therefore, damage to the embryo or foetus is unlikely when the MAK value is not exceeded and nitrilotriacetic acid and its sodium salts are assigned to Pregnancy Risk Group C. As genotoxicity has been observed only at or near cytotoxic doses, the substance is not regarded as a germ cell mutagen. The contribution of skin contact to systemic toxicity is expected to be relatively slight. Limited data show no sensitization.

MAK value (2019)	**2 mg/m³ I (inhalable fraction) as acid**
Peak limitation (2019)	**Category II, excursion factor 4**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity (2019)	**Category 4**
Prenatal toxicity (2019)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
CAS number	nitrilotriacetic acid: 139-13-9
	monosodium nitrilotriacetate: 18994-66-6
	disodium nitrilotriacetate: 15467-20-6
	disodium nitrilotriacetate monohydrate: 23255-03-0
	trisodium nitrilotriacetate: 5064-31-3
	trisodium nitrilotriacetate monohydrate: 18662-53-8
Molar mass	nitrilotriacetic acid: 191.14 g/mol
	trisodium nitrilotriacetate: 257.09 g/mol
Solubility	nitrilotriacetic acid: 1280 mg/l water at 22.5 ℃ (ECHA 2018 b, 2019)
	trisodium nitrilotriacetate: 457 g/l water at 20 ℃ (ECHA 2018 c)
log K_{OW}	nitrilotriacetic acid: –3.81 (calculated; ECHA 2018 b, 2019)
	trisodium nitrilotriacetate: –10.08 (calculated; ECHA 2018 c)
pKa value	nitrilotriacetic acid:
	at 20 ℃:
	pKa 1: 3.03
	pKa 2: 3.07
	pKa 3: 10.7 (ECHA 2018 b)
	at 25 ℃:
	pKa 1: 1.8
	pKa 2: 2.48
	pKa 3: 9.65 (ECHA 2019)
Vapour pressure	nitrilotriacetic acid: < 0.000001 hPa (ECHA 2019)
	trisodium nitrilotriacetate: 0.000000001 hPa at 25 ℃ (calculated; ECHA 2018 c)

Note: Avoid concurrent exposure to iron compounds (FeNTA formation)

Nitrilotriacetic acid (NTA) and its sodium salts were classified in Carcinogen Category 3 A in 2007 (Hartwig 2014). This supplement is a re-evaluation of this classification.

Since the documentation of 2008 (Hartwig 2014), only a few new studies have become available that are relevant to the evaluation. Only the toxicological end points investigated in these studies and those relevant for the re-evaluation of the carcinogenicity of the substance, the MAK value and the pregnancy risk group are evaluated in this supplement.

Mechanism of Action

Cytotoxicity

The severity of the nephrotoxic effects of orally administered nitrilotriacetic acid can be amplified by a higher supply of zinc in the diet; the NTA dose at which first effects occur is not affected (see Hartwig 2014 for more details).

Carcinogenicity

Kidneys and ureter

In vitro genotoxicity studies showed that in the range of 1 to 2 mM nitrilotriacetic acid, the concentration of essential calcium in the culture medium can be reduced by the formation of complexes, which induces clastogenic effects. There are numerous reasons which indicate a predominantly cytotoxic and not primarily genotoxic mechanism of action: the mainly negative results in the studies of genotoxicity; the wide range of toxic effects of nitrilotriacetic acid or nitrilotriacetic acid metal complexes on the kidneys and transitional epithelia of the ureter; the hyperplastic effects; the non-linear concentration–effect relationships (described by the law of mass action) for the formation of chelate complexes from nitrilotriacetic acid and divalent metal ions; and the exclusive occurrence of tumours in nephrotoxic dose ranges. The occurrence of numerous toxic effects in the kidneys is accompanied by an increase in zinc or zinc-NTA in the primary urine and urine (see Hartwig 2014 for more details).

Bladder

In addition to possible irritation by the crystals formed, the formation of tumours in the bladder is attributed to a possible decrease in the calcium in bladder tissue, as a reduced calcium level stimulates the basal cell mitogenesis of bladder tissue ex vivo (see Hartwig 2014 for more details).

Hyperplasia of the bladder epithelium can be regarded as an indicator of cytotoxic effects and thus as a precursor of tumours. The incidence of hyperplasia in female rats was significantly increased after nitrilotriacetic acid doses of 70 mg/kg body weight and day and above. Although hyperplasia of the bladder epithelium was observed in the males, no tumours were found. The reason for the sex-specific occurrence of the bladder tumours remains unexplained (see Hartwig 2014 for more details).

Significance of iron(III)-NTA (Fe(III)-NTA), copper-NTA and aluminium-NTA

In the case of oral administration, the formation of notable amounts of Fe(III)-NTA from NTA and nutritive or endogenous sources of iron in vivo can be excluded.

The available data also speak against the involvement of a copper-NTA complex in the effects of NTA or its sodium salts. And also in the case of aluminium-NTA, the available studies do not indicate the involvement of an aluminium-NTA complex in the effects of NTA or its sodium salts (see Hartwig 2014 for more details).

The kinetics of metal ions interacting with NTA was described in detail in the documentation from 2008 (Hartwig 2014).

Summary of the carcinogenic mechanism of action

Cytotoxic effects and possible genotoxicity (aneuploidy, clastogenicity) are regarded as the cause of tumour formation (Hartwig 2014).

As a result of their complex-forming properties, Na_3NTA and nitrilotriacetic acid can, however, reduce the concentration of divalent essential ions in the medium and thus presumably have indirect genotoxic effects. In addition, the alkalinity of Na_3NTA and the acidity of NTA may play a role in the positive in vivo findings observed. Positive findings were obtained in vitro at 1 mM nitrilotriacetic acid and above. Consequently, no genotoxicity is to be expected in vivo at concentrations below 1 mM nitrilotriacetic acid. The cytotoxic effects are therefore predominant as regards tumour formation. Ingested doses of nitrilotriacetic acid and their concentrations in urine and plasma correlate more or less linearly in rats up to Na_3NTA doses of 400 mg/kg body weight. Accordingly, an Na_3NTA dose of 15 mg/kg body weight corresponds in the rat to approximately 2 µM nitrilotriacetic acid in the plasma and 0.2 mM in the urine. Nitrilotriacetic acid concentrations at this level have no genotoxic effects in vitro (Hartwig 2014).

The new genotoxicity studies support the previously postulated mechanism of action, which states that the complex formation with calcium plays a role in the clastogenic effects at high doses. Since NTA is eliminated via the kidneys, there is presumably a deficiency in divalent cations, including calcium, which locally has indirect genotoxic effects (Nesslany et al. 2008).

Toxicokinetics and Metabolism

The oral absorption of disodium nitrilotriacetate in rats is practically 100%. This salt is also present at the pH of the rat stomach after the administration of NTA. It is eliminated with the urine and is not subject to enterohepatic circulation. The amount of NTA associated with the faeces in some studies was attributed by the authors to the incomplete separation of the urine and faeces. Oral absorption of NTA in mice is also complete. In rabbits, only 23% of orally given disodium nitrilotriacetate is eliminated with the urine and another 33% with the faeces. In humans, only 12% of the orally administered dose is eliminated with the urine and 77% with the faeces (Hartwig 2014; Michael and Wakim 1971).

In an in vitro study with human skin according to OECD Test Guideline 428, the dermal penetration of a 40% and a 1% Na_3NTA solution was investigated. The experiment with the 40% solution was terminated after 5 minutes because the severe skin damage resulting from the low pH greatly increased dermal penetration. The exposure to the 1% solution (skin load 100 µg/cm^2) took place over a period of 6 hours. From the experiment with the 1% solution, an absorption of 0.1% was determined (ECHA 2018 c). Assuming the exposure of 2000 cm^2 of skin for 1 hour to a still tolerable concentration of 38% (Hartwig 2014) would correspond to the absorption of 1.27 mg Na_3NTA (equivalent to 0.942 mg NTA).

For a saturated aqueous solution, the model of Fiserova-Bergerova et al. (1990) and the algorithm of the IH SkinPerm model (Tibaldi et al. 2014) calculate fluxes for NTA of 0.152 and 0.043 µg/cm^2 and hour, respectively. Assuming the exposure of 2000 cm^2 of skin for 1 hour, this would correspond to the dermal absorption of 304 and 86 µg, respectively.

Animal Experiments and in vitro Studies

Acute toxicity

Inhalation

No new studies with inhalation exposure have become available since the documentation of 2008 (Hartwig 2014).

Slight sensory irritation (parameters not defined) was observed at 220 mg Na_3NTA/m^3 after exposure of mice for 5 minutes. A NOAEC (no observed adverse effect concentration) was not obtained in this study. In a more recent study of sensory irritation in rats, an RD_{50} of 4300 mg/m^3 was derived (Hartwig 2014).

Subacute, subchronic and chronic toxicity

Inhalation

No new studies with inhalation exposure have become available since the documentation of 2008 (Hartwig 2014).

In an earlier, 4-week inhalation study with Na_3NTA in monkeys, an increase in respiration rate was described even at the lowest concentration tested of 10 mg/m³, but the method described is inadequate and definitive conclusions concerning the effects of sensory irritation cannot be drawn (Hartwig 2014). The study can therefore not be used for the derivation of a MAK value.

Reproductive and developmental toxicity

Developmental toxicity

No new studies of developmental toxicity have become available since the documentation of 2008 (Hartwig 2014).

An earlier feeding study in rats over two generations with Na_3NTA doses of up to 450 mg/kg body weight and day (NTA doses of 335 mg/kg body weight and day) yielded no evidence of either impairment of fertility or embryotoxic effects. Earlier studies of developmental toxicity provided no evidence of adverse effects of nitrilotriacetic acid or Na_3NTA in the offspring of rats up to the highest Na_3NTA dose tested of about 450 mg/kg body weight and day (nitrilotriacetic acid doses of about 335 mg/kg body weight and day), or in the offspring of rabbits up to nitrilotriacetic acid doses of 250 mg/kg body weight and day, or in the offspring of mice at the only nitrilotriacetic acid dose tested of about 400 mg/kg body weight and day. No maternal toxicity occurred at the doses tested.

Genotoxicity

The available data for genotoxicity do not indicate that nitrilotriacetic acid itself causes genotoxic effects (Hartwig 2014).

A publication released in the meantime includes several genotoxicity tests (Nesslany et al. 2008), which are described below.

In vitro

The studies were conducted with NTA dissolved in aqueous sodium hydroxide, and therefore actually with a sodium salt of NTA.

A mutagenicity test with the Salmonella typhimurium strains TA98, TA100, TA102 and TA1537 at concentrations of up to 5000 µg NTA/plate with and without the addition of a metabolic activation system (S9 mix from rat liver or kidney) yielded negative results. When kidney S9 mix was added, NADP was added in the first test and replaced by arachidonic acid in the second test. This cofactor was used because prostaglandin synthase and/or lipoxygenase have been shown to favour in vivo DNA adduct formation in the kidneys by some mutagens (Nesslany et al. 2008).

In the comet assay in L5178Y mouse lymphoma cells, NTA concentrations of 312.5 to 2500 µg/ml induced a marked, statistically significant increase in the medians of the olive tail moments (OTMs) to values of 2.35 to 5.59 after 4 hours of incubation, compared with 1.01 in the control. To investigate whether this result is affected by blocking the complex/chelate-forming activity of NTA, equimolar amounts of Ca^{2+} were added to the test system. The induction of DNA damage was then no longer observed under the same test conditions; there was even a statistically significant decrease in DNA damage. The addition of EDTA alone, on the other hand, likewise led to an increase in DNA damage (Nesslany et al. 2008).

In the comet assay in primary kidney cells from male Sprague Dawley rats, NTA induced a statistically significant increase in DNA damage after a 4-hour incubation period at the three lowest concentrations of 156.25 to 625 µg/ml. After the addition of equimolar amounts of Ca^{2+}, however, a statistically significant increase in the medians of the OTMs occurred at the three middle concentrations of 312.5 to 1250 µg/ml. Treatment with EDTA or Ca^{2+} alone induced DNA damage over the entire concentration range tested (Nesslany et al. 2008).

A micronucleus assay in L5178Y mouse lymphoma cells was likewise performed with and without the addition of a metabolic activation system from rat liver or kidney and with the addition of kidney S9 mix with NADP or arachidonic acid with NTA at concentrations of up to 2500 µg/ml. The cells were incubated for 24 hours (without metabolic activation) or 4 hours (with metabolic activation). NTA increased the number of micronucleated cells, with concomitant moderate cytotoxicity (> 50% relative survival), only without metabolic activation. At concentrations of 625 and 1250 µg/ml, the incidence of cells with micronuclei increased to 15 and 10 per 2000 cells, respectively, compared with 2 in the controls. A 50% relative cell survival rate is considered acceptable for the assessment of genotoxic effects. At 312.5 µg/ml and 84.9% relative survival, micronuclei were not induced. After a 20-hour recovery period, increased numbers of micronucleated cells were observed at the cytotoxic concentrations of 625 and 1250 µg/ml (of 2000 cells examined, 21 and 60 cells with micronuclei, respectively; in the control 5). Equimolar concentrations of Ca^{2+} were added to the test system to block the complex/chelate-forming activity of NTA. The induction of micronuclei was then no longer observed under the above conditions (Nesslany et al. 2008).

Another micronucleus assay coupled with the detection of apoptosis was performed with CTLL2 and CTLL2/Bcl2 cells. CTLL2 is a stable subclone of cytotoxic T lymphocytes, originally isolated from the C57BL/6 mouse. The CTLL2/Bcl2 cell line was generated by stable transfection with the pSFFV neo bcl2 plasmid. Neither an increase in micronuclei nor in apoptosis occurred with the addition of liver or kidney S9 mix and at NTA concentrations of 0, 625, 1250 or 2500 µg/ml. Without the addition of S9 mix NTA did induce a statistically significant increase in micronuclei in both cell lines, but no increase in apoptosis. In CTLL2 cells, after treatment for 24 hours without a recovery period, there were 15 and 50 micronuclei at the two high concentrations of 1250 and 2500 µg/ml, respectively, compared with 4 in the control (2000 cells examined in each case). In CTLL2/Bcl2 cells, an increased number of micronuclei was observed only at 2500 µg/ml with an incidence of 26 (3 in the control). These genotoxic concentrations induced no to moderate cytotoxicity. After a recovery time of 20 hours, increased incidences of micronuclei occurred in both cell lines at 2500 µg/ml (29 and 26 cells with micronuclei compared with 5 and 6, respectively, in the control) (Nesslany et al. 2008).

In a $TK^{+/-}$ mutation assay with L5178Y mouse lymphoma cells, the mutation frequency after continuous treatment of the cells with NTA for 24 hours was significantly increased compared with the spontaneous mutation frequency. The maximum response was observed at the highest analysable concentration of 444.4 µg/ml (adjusted relative total growth of 33.5%) with an induction ratio of 1.9. Induction ratios of < 2 are not considered biologically significant. Higher concentrations led to induction ratios of > 2, accompanied by marked cytotoxicity with relative cell growth of < 10%. The induction ratio of small colonies (indicative of chromosomal aberrations) was 2.5 at 444.4 µg/ml. A second assay, with a narrower range of NTA concentrations, yielded a dose-dependent increase in the total induction ratios (2.5 and 4.1) and in the induction ratio of small colonies (2.9 and 4.7) at 450 and 500 µg/ml (relative cell growth 31.1% and 10.2%, respectively). The highest concentration tested of 550 µg/ml was not included in the evaluation due to cytotoxicity (relative cell growth 8.9%). In view of the results of the previous genotoxicity tests, testing was performed only without the addition of a metabolic activation system (Nesslany et al. 2008).

A chromosomal aberration assay with human lymphocytes was performed after 4 hours of treatment with NTA and a further 16 hours of growth (1.5-fold cell cycle length), and after 20 and 44 hours of treatment (1.5 and 2.5-fold cell cycle length, respectively) with immediate analysis of the metaphases. Human lymphocytes were obtained from male, healthy, non-smokers (< 40 years), cytotoxicity was determined by mitotic index. Two hours before harvesting, colcemid was added to the cell cultures and at the end of the assay 200 metaphases from each culture were examined for chromosomal changes: structural aberrations (gaps, breaks, triradial and tetraradial exchanges) and numerical aberrations (aneuploidy and polyploidy). On the basis of the results of the previous tests, no S9 mix was added. After treatment for 4 hours, there was an increase in aberrant cells, but this was not concentration-dependent and not statistically significant. After exposure for 20 hours, however, a statistically significant and concentration-dependent

increase in chromosomal breaks was observed at 1000 to 2000 μg/ml. After treatment for 44 hours, the induction of aberrations was statistically significant, but not concentration-dependent. Likewise after treatment for 44 hours, the frequency of polyploid cells was increased, but not statistically significant. An induction of interchromosomal or intrachromosomal exchanges was not observed at any of the treatment times (Nesslany et al. 2008).

In vivo

After the administration of single oral NTA doses of 0, 1000 or 2000 mg/kg body weight to groups of 5 male Sprague Dawley rats, a comet assay was performed with the kidney cells after 3 to 6 and 22 to 26 hours. NTA induced a marked, statistically significant increase in DNA damage at both doses, but this was not dose-dependent. The median OTMs after the administration of 0, 1000 and 2000 mg NTA/kg body weight were 6.31, 20.09 and 16.81, respectively, after 3 to 6 hours and 4.30, 19.38 and 16.80, respectively, after 22 to 26 hours. In a preliminary study, neither mortality nor clinical signs were observed after treatment with 2000 mg/kg body weight. A NOAEL (no observed adverse effect level) was not obtained (Nesslany et al. 2008).

Summary

The data for genotoxicity do not provide evidence of genotoxic effects by NTA itself (Hartwig 2014).

Indirect genotoxic effects such as disturbed chromosomal distribution are due to the chelating properties of NTA which leads to a disturbance of the spindle apparatus (Nesslany et al. 2008).

The new genotoxicity studies in vitro and in vivo support the previous data. They also yield evidence of indirect clastogenic effects that occur only at or just below cytotoxic doses. Positive results were obtained only without the addition of a metabolic activation system (Nesslany et al. 2008).

Carcinogenicity

In long-term studies in rats and mice, tumours were found in the kidneys and urinary tract after oral administration of NTA and its sodium salts with the drinking water or feed (Hartwig 2014).

In a number of tumour-promotion studies, nitrilotriacetic acid (after initiation with nitrosamines) was found to be a tumour promotor in the kidneys and bladder of rats and mice (Hartwig 2014).

In rats, renal tubular carcinomas and transitional cell carcinomas of the kidney and urinary tract occurred at and above 100 mg Na_3NTA/kg body weight and day (74 mg NTA/kg body weight and day), in mice at and above 1125 mg NTA/kg body weight and day. At Na_3NTA doses of 38 mg/kg body weight and day (27 mg NTA/kg body weight and day) and above, cytotoxic effects in the kidneys of rats and an increase in the concentration of zinc in the urine of dogs were observed. The NOAEL for increased zinc excretion in rats was 9 mg Na_3NTA/kg body weight and day (6.7 mg NTA/kg body weight and day) or 15 mg Na_3NTA/kg body weight and day (11.2 mg NTA/kg body weight and day) and for dogs 8 mg NA_3NTA/kg body weight and day (5.6 mg NTA/kg body weight and day) (Hartwig 2014).

The incidence of hyperplasia of the bladder epithelium was increased in male rats at 10 mg Na_3NTA/kg body weight and day (6.9 mg NTA/kg body weight and day; 3 of 23 animals; not statistically significant) and in female rats at 70 mg NTA/kg body weight and day and above (statistically significant). Bladder tumours were observed mainly in female rats at 260 mg NTA/kg body weight and day and above (Hartwig 2014).

Manifesto (MAK value/classification)

The critical effect is the carcinogenicity of the substance in the kidney and urinary tract.

MAK value. Inhalation studies from which a MAK value can be derived are not available.

The lowest LOAEL (lowest observed adverse effect level) for systemic effects (see Table 1) comes from a 2-year feeding study in rats and is 10 mg Na_3NTA/kg body weight and day (6.9 mg NTA/kg body weight and day). Here, hyperplasia of the bladder epithelium occurred in 3 of 23 animals (Hartwig 2014 and Section "Carcinogenicity"). Since no NOAEL was obtained, a NAEL (no adverse effect level) of 2.3 mg NTA/kg body weight and day corresponding to one third of the LOAEL has been estimated. The following toxicokinetic data are taken into consideration for the extrapolation of this NAEL to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding species-specific correction value for the rat (1:4), the measured oral absorption of 100% (Hartwig 2014), the body weight (70 kg) and respiratory volume (10 m³) of the person, and the assumed 100% absorption by inhalation. The concentration calculated from this is 5.6 mg NTA/m³. As this value comes from a NAEL from experimental studies with animals (1:2), a MAK value of 2 mg NTA/m³ I (inhalable fraction) can be derived using the preferred value approach.

Tab. 1 Effect levels (LOAELs) of NTA and Na_3NTA in comparison (Hartwig 2014)

Species	Findings		$Na_3NTA \times H_2O$	Na_3NTA	NTA
			(mg/kg body weight and day)[a]		
mouse	carcinomas	kidneys			1125
rat	carcinomas	kidneys		100	74
rat	cytotoxicity	kidneys		38	27
rat (female)	carcinomas	bladder	375		258
rat (female)	hyperplasia	bladder	100		69
rat (male)	hyperplasia (not statistically significant)	bladder	10		6.9 **NAEL 2.3**
rat	increased zinc concentration	urine		38 NOAEL 9	27 NOAEL 6.7
rat	increased zinc concentration	urine			342 mg/m³ NOAEC 213 mg/m³
rat	genotoxicity in vitro at 1 mM NTA and above	1 mM in the urine corresponding to:	80	75	56

[a] unless otherwise stated

There is an adequate margin between the dose from which the MAK value was derived (2.3 mg NTA/kg body weight and day) and the lowest dose at which renal tumours occurred in rats (see Table 1) of 100 mg Na_3NTA/kg body weight and day (corresponding to 74 mg NTA/kg body weight and day) (Hartwig 2014 and Section "Carcinogenicity").

Irritation is not to be expected with exposure at the level of the MAK value because NTA is not corrosive and for corrosive acids such as citric acid a MAK value of 2 mg/m³ I has been set.

The same MAK value can be established also for the sodium salts calculated as NTA. Irritation is not to be expected here either, since the RD_{50} for Na_3NTA is 4300 mg/m³ and the sodium salts are not corrosive.

In addition, accumulation of the substance in the lungs is not to be expected due to its good water solubility.

A 90-day inhalation study in rats with Na_2H_2EDTA can be used to answer the question as to what effects a chelating agent such as NTA has on the respiratory tract. Na_2H_2EDTA is a chelating agent with significantly stronger affinity to Ca^{2+} and Zn^{2+} ions than NTA. Effects on the lung (alveolar histiocytosis grade 1) and on the epiglottis (adaptive epithelial changes grade 1–2, squamous metaplasia grade 1) as well as an increase in absolute and relative lung weights in the female animals were observed in the 90-day study with rats at 3 mg/m³. The authors of the study reported 3 mg/m³ to be the NOAEC, and 15 mg/m³ the LOAEC (lowest observed adverse effect concentration) (ECHA 2018 a).

EDTA binds calcium ions at pH 7 about 4000 times more strongly than NTA, zinc ions about 40 000 times more strongly:

Conditional complexation constants for NTA:

$\log K$ (Ca^{2+}) = 2.6; $\log K$ (Zn^{2+}) = 7.9 (Hartwig 2014).

Conditional complexation constants for Na_2H_2EDTA:

$\log K$ (Ca^{2+}) = 6.2; $\log K$ (Zn^{2+}) = 12.5 (BASF SE 2018).

At a MAK value of 2 mg NTA/m³ I, therefore, no local cytotoxicity in the respiratory tract is to be expected because local effects of EDTA, a significantly *stronger* chelator than NTA, are first observed at 3 mg/m³.

Simultaneous inhalation of iron compounds chelatable by NTA should be avoided because of the possibility of the formation and absorption of FeNTA.

Peak limitation. The MAK value is derived from a systemic effect. NTA has therefore been assigned to Peak Limitation Category II. The half-life of NTA in humans is about 4 hours (Hartwig 2014). Therefore, an excursion factor of 4 has been set for nitrilotriacetic acid and its sodium salts (Hartwig and MAK Commission 2017).

Prenatal toxicity. In the developmental toxicity studies, there was no evidence of adverse effects of NTA or Na_3NTA on the foetuses of rats up to the highest dose tested of about 450 mg Na_3NTA/kg body weight and day (corresponding to 335 mg NTA/kg body weight and day) or on the foetuses of rabbits at doses of up to 250 mg NTA/kg body weight and day, or on those of mice at the only dose level tested (400 mg NTA/kg body weight and day). Maternal toxicity did not occur at these dose levels.

The following toxicokinetic data are taken into consideration for the extrapolation of the NOAELs of 335 mg/kg body weight and day in the rat, 250 mg/kg body weight and day in the rabbit and 400 mg/kg body weight and day in the mouse to a concentration in workplace air: the corresponding species-specific correction values for the rat, the rabbit and the mouse (1:4, 1:2.4 and 1:7, respectively), the measured oral absorption of 100% for rats and mice and 23% for rabbits, the body weight (70 kg) and respiratory volume (10 m³) of the person, and the assumed 100% absorption by inhalation. The concentrations calculated from these data are 586, 168 and 400 mg/m³, respectively. Since the 293-fold, 84-fold and 200-fold margins between the calculated NOAECs to the MAK value of 2 mg/m³ are sufficiently large, nitrilotriacetic acid and its sodium salts have been assigned to Pregnancy Risk Group C.

Carcinogenicity. In long-term studies in rats and mice, tumours were found in the kidney and urinary tract after oral administration of NTA and its sodium salts with the drinking water or feed. In studies in rats and mice, tumour-promoting effects of NTA on the kidney and bladder have been demonstrated after initiation with nitrosamines.

For renal toxicity, a NOAEL of 15 mg Na_3NTA/kg body weight and day was obtained in rats. The lowest dose at which renal tumours occur in rats is 100 mg Na_3NTA/kg body weight and day (corresponding to 74 mg NTA/kg body weight and day) (Hartwig 2014).

However, the bladder seems to be more sensitive; here the LOAEL is 10 mg Na_3NTA/kg body weight and day (6.9 mg NTA/kg body weight and day). At this dose, hyperplasia of the bladder epithelium was reported in 3 of 23 rats in the long-term study. Hyperplasia of the bladder epithelium is to be regarded as an indicator of cytotoxicity and thus as a precursor of tumours. In female rats, the increase in such hyperplasia was statistically significant at 70 mg NTA/kg body weight and day and above, bladder tumours were found at 260 mg NTA/kg body weight and day and above (Hartwig 2014).

Due to the mechanistically well-established dose range without genotoxic and cytotoxic effects, without increased cell proliferation in the kidneys and without significant formation of NTA metal complexes, NTA and its sodium salts have been assigned to Carcinogen Category 4.

Germ cell mutagenicity. The new genotoxicity studies in vitro and in vivo support the previous data. They also indicate indirect clastogenic effects occurring near or at cytotoxic doses. NTA has therefore not been classified in one of the categories for germ cell mutagens.

Absorption through the skin.   Experimental data for skin penetration are available for Na$_3$NTA but not for NTA.

For humans, the dermal absorption of a maximum amount of 1.27 mg Na$_3$NTA (equivalent to 0.942 mg NTA) can be estimated from an in vitro study with Na$_3$NTA (Section "Toxicokinetics and Metabolism") following exposure to a 38% non-irritant solution under standard conditions (2000 cm^2 of skin, exposure for 1 hour).

A model calculation (Section "Toxicokinetics and Metabolism") resulted in the dermal absorption of a maximum amount of 0.304 mg for NTA with exposure to a saturated aqueous solution under standard conditions (2000 cm^2 of skin, exposure for 1 hour).

For the monosodium and disodium salts, the amounts absorbed can be assumed to be between those for the trisodium salt and the acid.

However, 8-hour inhalation exposure at the level of the MAK value of 2 mg/m^3 would result in the absorption of 20 mg, assuming 100% absorption by inhalation and a respiratory volume of 10 m^3. Consequently, the estimated amounts dermally absorbed of both NTA and Na$_3$NTA are so low that the MAK value provides sufficient protection against systemic effects even in the case of additional dermal contact. This applies also to the other sodium salts. Therefore, NTA and its sodium salts are not designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization.   There are still no findings of sensitizing effects in humans and no positive results from experimental studies in animals or in vitro studies. Nitrilotriacetic acid and its sodium salts are therefore not designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

References

BASF SE (2018) Communication from Dr. Edgar Leibold. Email, 16 Oct 2018

ECHA (European Chemicals Agency) (2018 a) Disodium dihydrogen ethylenediaminetetraacetate (CAS Number 139-33-3). Registration dossier. Joint submission, first publication 20 Dec 2010, last modification 03 Aug 2018. https://echa.europa.eu/registration-dossier/-/registered-dossier/14817, accessed 15 Oct 2018

ECHA (European Chemicals Agency) (2018 b) Nitrilotriacetic acid (CAS Number 139-13-9). Registration dossier. Joint submission, first publication 02 Mar 2011, last modification 18 May 2018. https://echa.europa.eu/registration-dossier/-/registered-dossier/11946, accessed 08 Oct 2018

ECHA (European Chemicals Agency) (2018 c) Trisodium nitrilotriacetate (CAS Number 5064-31-3). Registration dossier. Joint submission, first publication 17 Mar 2011, last modification 19 Feb 2018. https://echa.europa.eu/registration-dossier/-/registered-dossier/14301, accessed 08 Oct 2018

ECHA (European Chemicals Agency) (2019) Nitrilotriacetic acid (CAS Number 139-13-9). Registration dossier. Joint submission, first publication 17 May 2013, last modification 14 May 2019. https://echa.europa.eu/registration-dossier/-/registered-dossier/12171, accessed 22 May 2019

Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. Am J Ind Med 17(5): 617–635. https://doi.org/10.1002/ajim.4700170507

Hartwig A, editor (2014) Nitrilotriacetic acid and its sodium salts. MAK Value Documentation, 2008. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Weinheim: Wiley-VCH. https://doi.org/10.1002/3527600418.mb13913vere4514

Hartwig A, MAK Commission (2017) Peak limitation: Limitation of exposure peaks and short-term exposures. MAK Value Documentation, 2011. MAK Collect Occup Health Saf 2(1): 2–6. https://doi.org/10.1002/3527600418.mbpeakexpe5117

Michael WR, Wakim JM (1971) Metabolism of nitrilotriacetic acid (NTA). Toxicol Appl Pharmacol 18(2): 407–416. https://doi.org/10.1016/0041-008x(71)90133-5

Nesslany F, Simar-Meintières S, Watzinger M, Talahari I, Marzin D (2008) Characterization of the genotoxicity of nitrilotriacetic acid. Environ Mol Mutagen 49(6): 439–452. https://doi.org/10.1002/em.20403

Tibaldi R, ten Berge W, Drolet D (2014) Dermal absorption of chemicals: estimation by IH SkinPerm. J Occup Environ Hyg 11(1): 19–31. https://doi.org/10.1080/15459624.2013.831983