

RISK ASSESSMENT

Trisodium Nitrilotriacetate

CAS-No.: 5064-31-3

EINECS-No.: 225-768-6

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Information on the rapporteur

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The first draft of the Human Health Section of the Comprehensive Risk Assessment Report Trisodium Nitrotriacetate (NTA), a substance chosen from the EU 3rd Priority List in 1995, which is distributed for the preliminary written procedure was distributed to MS for preliminary comments in October 2006.

This document is the revised draft of the Human Health Section of the RAR of NTA which is intended to be discussed in-depth at the TC NES II'07.

Contents

0	OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT	4
1	GENERAL SUBSTANCE INFORMATION	7
2	GENERAL INFORMATION ON EXPOSURE	11
2.1	Production, Import and Export	11
2.2	Processing / Application (Categories of use, Amounts)	11
2.3	Use Pattern	13
3	ENVIRONMENT	14
3.1	Environmental exposure	14
3.1.1	General discussion	14
3.1.2	Aquatic compartment	24
3.1.3	Atmosphere	36
3.1.4	Terrestrial compartment	37
3.1.5	Non compartment specific exposure relevant to the food chain	37
3.2	Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment	37
3.2.1	Aquatic compartment	37
3.2.2	Atmosphere	54
3.2.3	Terrestrial compartment	54
3.2.4	Non compartment specific effects relevant to the food chain	55

3.3	Risk characterisation	55
3.3.1	Aquatic compartment	55
3.3.2	Atmosphere	56
3.3.3	Terrestrial compartment	56
3.3.4	Non compartment specific effects relevant to the food chain	56
4	HUMAN HEALTH	57
4.1	HUMAN HEALTH (TOXICITY)	57
4.1.1	Exposure assessment	57
4.1.2	Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment	83
4.1.3	Risk characterisation	167
4.2	HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)	199
4.2.1	Exposure assessment	199
4.2.2	Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment	199
4.2.3	Risk characterisation	199
5	CONCLUSIONS / RESULTS	201
6	REFERENCES	203
	APPENDIX 1	217

0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No.	5064-31-3
EINECS No.	225-768-6
IUPAC Name	Trisodium nitrilotriacetate

Overall results of the risk assessment:

- i) There is need for further information and/or testing
- ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already
- iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusions:

Environment

Conclusion ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

In the present risk assessment production and use of Na₃NTA are examined. For all life-cycle steps, the PEC/PNEC ratios are below 1. Therefore, a risk for the environment is not expected.

Human Health

Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Concern is derived for repeated dose toxicity and carcinogenicity. Especially dermal exposure (with a critical exposure level of 2.85 mg/kg/day) has to be reduced for scenario 2 (use of Na₃NTA in formulation process) and 3 (high pressure cleaning), and inhalation exposure (with a critical exposure level of 2 mg/m³) has to be reduced for scenario 1 (production of NTA) and 2b (use of Na₃NTA in formulation process without LEV).

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

For the other toxicological endpoints the risk orientated conclusions result in no concern with the consequence that risk reduction measures are of low priority.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

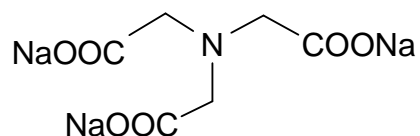
Man exposed indirectly via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

1 GENERAL SUBSTANCE INFORMATION

Identification of the substance

CAS-No.:	5064-31-3
EINECS-No.:	225-768-6
IUPAC Name:	Trisodium nitrilotriacetate
Synonyms:	Nitrilotriacetic acid trisodium salt NTA trisodium salt
CA-Index name:	Glycine, N,N-bis(carboxymethyl)-, trisodium salt
Molecular weight:	257.1 g/mol
Empirical formula:	C ₆ H ₆ NNa ₃ O ₆
Structural formula:	



Purity/impurities, additives

Purity:	≥ 92 % w/w
Impurities:	< 7 % water < 3 % sodium glycolate < 2 % disodium iminodi(acetate) < 2 % sodium hydroxide < 1.5 % methanamine < 1 % sodium formate
Additives:	none

Physico-chemical properties

Trisodium nitrilotriacetate (Na₃NTA) is a colourless crystalline powder at room temperature and normal pressure. Data on the physical and chemical properties are given in table 1.1.

Table 1.1: Data on the physical and chemical properties of Na₃NTA

Melting point	410 °C with decomposition above 200 °C	BASF, 1996
Boiling point	not applicable	
Relative density	1.77 at 20 °C ¹⁾	BASF, 1997
Vapour pressure	not determined	no test conducted because of structural reasons
Surface tension	not determined	no test conducted because of structural reasons
Water solubility	about 640 g/l at 20 °C	Ullmann, 1991
Partition coefficient	-2.62 (calculated) ²⁾	BASF, 1989
Flash point	not determined	substance is a solid
Flammability	not highly flammable ³⁾	BASF, 1998
Ignition temperature	no selfignition up to decomposition (200 °C)	Chemsafe, 1997
Explosive properties	not explosive	no test conducted because of structural reasons
Oxidising properties	no oxidising properties	no test conducted because of structural reasons

¹⁾ Relative density: pycnometer method

²⁾ Partition coefficient: The logPow was calculated according to the Rekker method with the computer program Pro-logP (version 2.0).

³⁾ Flammability: According to guideline 92/69/EEC test method A.10 (determination of the flammability for solids), the substance did not propagate combustion. The tests according to A.12 (determination of the flammability in contact with water) and A.13 (determination of pyrophoric properties) were not conducted. Due to the properties

and the handling of the substance it has not to be assumed that flammable gases formate in contact with water or the substance has pyrophoric properties.

Classification

Environment

Proposal of the rapporteur (only environmental part)

For the classification of biodegradation, the available laboratory tests with uncomplexed NTA should not be used, because biodegradation of this chelator is strongly dependent on the metal speciation. Studies on the degradation in biological treatment plants as well as degradation tests conducted in river water reveal that the degradation properties of NTA (resp. of its metal complexes) are comparable with a readily degradable substance. In addition the substance has no bioaccumulation potential.

In tests on the acute toxicity on fish and daphnia effects were only observed when NTA was present in over-stoichiometric concentrations compared to the content of metal ions. The lowest LC50 values were 98 mg/l for both trophic levels.

Results of algae growth inhibition tests have to be interpreted carefully, because the observed effects are mainly cause by nutrient deficiency, which is an artefact and not relevant for the environment. Tests with increased concentrations of nutrient metals (where nutrient deficiency is suppressed) reveal that intrinsic toxicity of NTA is expected only at concentrations far above 10 mg/l.

Considering all results, a classification of Na₃NTA is not recommended with respect to the environment.

Human Health

- (Classification according to Annex I)

Xn	Harmful	R 22	Harmful if swallowed
Xi	Irritant	R 36	Irritating to eyes

- (Proposal of the rapporteur)

Classification and Labelling of Na₃NTA has been concluded by the EU C&L Committee in March 2006 (See ANNEX 1 Draft list 31st ATP New entries 16/10/2006 (31B)).

Xn	Harmful	R 22	Harmful if swallowed
Xi	Irritant	R 36	Irritating to eyes

Carcinogenic, cat. 3 R 40 Limited evidence of a carcinogenic effect

Oral LD50 values of approximately 750 mg/kg bw were determined for monkeys, of 1300-1470 mg/kg bw for female rats and of 1600-2220 mg/kg bw for male rats. Based on these data the substance has to be classified as "Xn, harmful" and labelled with "R 22 - harmful if swallowed".

There is no valid information on eye irritation testing according to current international test guidelines. On animal welfare reasons, we propose to assess the eye irritating properties of Na₃NTA on the basis of the test carried out for Monsanto (1968) and BASF (1982) and taking also into account the strongly basic nature of a 1% aqueous solution. The Monsanto test reports on conjunctival irritation and corneal effects which were not reversible within 7 days. However, based on the nature of effects, reversibility is to be expected within an observation time of 21 days. Correspondingly, effects of a 38% aqueous solution were moderate (BASF 1982). These results are sufficient to confirm "R 36 irritating to eyes".

Carcinogenicity of NTA was demonstrated for the oral route. NTA is carcinogenic in both sexes of two species, the rat and the mouse. No or sparse data were evident for the other inhalative or dermal routes. NTA is not metabolised and exerts its carcinogenic activities via the urinary excretion route. NTA induced primary tumors at several localisations in the urinary tract. Multiple tumor types were observed. Tumors in the rat kidney originated from the tubular-cell epithelium and from the pelvic transitional cell epithelium. Tumors from the transitional cell type were also found in the ureter and the urinary bladder. For the mice, tumors originated from the renal tubular epithelium and occasionally from the renal pelvis.

Based on the actual knowledge a direct genotoxic mechanism of NTA carcinogenesis could not be demonstrated. At present, it is thought that NTA might be operative at target sites by other actions. It seems to be reasonable that NTA related cytotoxicity plays a crucial role in the development of tumors. The facts that the cytotoxicity and tumors were seen in identical target regions of the kidneys, that cytotoxicity is an early lesion that leads to regenerative hyperplasia and that hyperplasia was often associated to tumor growth provide evidence for this mode of action, which is in line with the criteria for a carcinogen, category 3. .

Beside the sequential cascade of morphological events – cytotoxicity, hyperplasia/dysplasia, neoplasia – other factors might contribute to disregulated cell growth and to the manifestation of neoplastic cell growth either as initial events before obvious cytotoxicity or in parallel to the cascade from cytotoxicity to tumor. Interference with metal cations and forming of metal complexes might be suspected. However, the data presently available were insufficient to give sufficient evidence for their contribution in the NTA carcinogenicity.

Concentration Limits

$C \geq 25 \%$: Xn; R22-36-40

$20 \leq \% C < 25 \%$: Xn; R36-40

$5 \leq \% C < 20 \%$: Xn; R40

2 GENERAL INFORMATION ON EXPOSURE

Premark:

NTA is produced and used as sodium salt (Na₃NTA) or as acid (H₃NTA). During the use and, after release into the environment, complexes with metal ions are formed. The environmental exposure of all NTA complex species is overlapping. In the scientific literature dealing with the environmental fate and toxic effects on organisms, amounts and concentrations are mostly referred as Na₃NTA. Thus, for the environmental risk assessment (sections 2 and 3) all production and use volumes are given as Na₃NTA equivalents.

2.1 PRODUCTION, IMPORT AND EXPORT

The following companies are producer and/or importers of NTA:

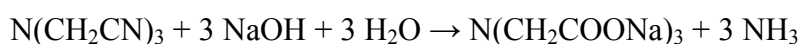
- Akzo Nobel Chemicals B.V., Herkenbosch (NL)
- Akzo Nobel Chemicals B.V., Kvantorp (SWE)
- BASF AG, Ludwigshafen (GER)
- Dow Europe S.A., Seal Sands (UK)
- Solutia Europe S.A. (BEL)

According to the data supplied by the producers and importers for this report, 36090 t/a (calculated as Na₃NTA) are produced, 6040 t/a are imported and 10090 t/a exported outside of the EU, thus 32040 t/a are consumed within Europe. According to CEFIC (2001), 26642 t/a were marketed in 2000, the difference to the producers data might be explained by exports or imports of NTA containing formulations.

2.2 PROCESSING / APPLICATION (CATEGORIES OF USE, AMOUNTS)

Today the original synthesis of NTA from ammonia and chloroacetic acid has only historical significance. The oxidation of triethanolamine is likewise of no industrial importance. The one-stage alkaline and two-stage acid processes now in use are based on the cyanomethylate ion of ammonia (or ammonium sulphate) with formaldehyde and sodium cyanide (or hydrogen cyanide).

The alkaline process was long the established method for NTA production. Trisodium nitrilotriacetate is synthesized as follows:



The reaction can be carried out batch wise or continuously, but the continuous process is more economical. The resulting solution is sold directly as a 40-wt% solution, or used in the production of Na₃NTA in powder form, or acidified to pH 1 - 2 to yield the acid (H₃NTA).

Acid Process: The significant yield of by-products in the alkaline process has led in recent years to the construction of plants based on the acid process, which features much lower by-product levels. The acid process is associated with stringent safety requirements due to the use of hydrogen cyanide; corrosion can also be a problem. In the first stage, ammonia is reacted with formaldehyde to give hexamethylenetetramine, which is then reacted with hydrogen cyanide in sulphuric acid solution to yield triscyanomethyl amine. The solid triscyanomethyl amine is sparingly soluble in the acidic solution and is filtered off, washed, and saponified with NaOH to give Na₃NTA. The resulting solution has a far lower by-product content than the solution from the alkaline method. It is also sold as 40 % product or used in the production of Na₃NTA or H₃NTA (see above).

The NTA amounts (calculated as Na₃NTA) marketed in the Western European countries are given in the following table. The figures are derived from sales information of the producers. A direct correlation to the consumption volume is therefore not precise, however the figures may be regarded as an approximation for the European consumption. Imports and exports of Na₃NTA containing formulations are not considered.

Table 2.2: Na₃NTA Sales in European Countries (CEFIC, 2000)

Country	Sales [t] in 1999
Germany	3396
Belgium / Luxembourg	1814
The Netherlands	2055
France	1838
Italy	825
UK	7274
Ireland / Denmark	561
Spain / Portugal / Greece	5108
Finland	Not published
Norway	0
Sweden	1734
Austria	Not published
Switzerland	631
Total West. Europe	26756

2.3 USE PATTERN

NTA is an aminocarboxylic acid with three functional groups which donate electrons. These enable it to participate in complexation reactions. The most important property of NTA is to form water-soluble complexes with multivalent metal ions over a wide pH range.

NTA and its sodium salt are used to soften water and to remove traces of alkaline earth and heavy metals. They are often included in detergent and cleaner formulations for household or industrial use.

The application volumes (calculated as Na₃NTA) were (CEFIC, 2000; CEFIC 2001):

Table 2.3: Use pattern of Na₃NTA (CEFIC, 2000; CEFIC 2001)

	IC/UC *	Germany (1999)	Western Europe (2000)
Marketed amount		3396 t	26642 t
Textile cleaning, household and industrial	5 / 11 13 / 11	238 t (7%)	973 (3.7%)
Cleaning agents	6 / 9	2207 (65%)	17905 (67.2%)
Others	15 / 0	951 (28%)	7764 (29.1%)

* industrial category / use category

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

3.1.1.1 Release into the Environment

Production

During production, releases occur via waste water into the hydrosphere. According to the data submitted by the producers, the total yearly releases into the hydrosphere are 24.1 t/a (cf. 3.1.2.1).

Use

During the use as complexing agent, the major amount of the applied NTA is released into the waste water. The emission situation for the individual uses is presented in section 3.1.2.2.

Frequently the question is raised in the literature whether NTA can cause hazardous effects due to its property to keep heavy metal ions in the water phase of rivers. The interaction of NTA with metal ions is elaborated in section 3.1.2.3.

3.1.1.2 Degradation

3.1.1.2.1 Biodegradation

Laboratory biodegradation tests

A series of laboratory degradation tests is available for NTA (Table 3.1). In most cases the acid or the Na-salt was added and not the complexed NTA. However, the test media generally contain, beside trace metals, calcium and/or magnesium ions in over-stoichiometric amounts, the respective complexes are formed thus being the active test substances.

The test results indicate that the Ca/Mg complexes are readily removed. For instance, in a Modified OECD Screening Test conducted according to OECD guideline 301 E, NTA was found to be readily biodegradable. Meeting the 10 days time window criterion, the substance (initial concentration 50 mg/l) was completely degraded within 14 days, as measured by DOC. The inoculum used was taken from river water treatment plant. The lag phase until degradation started was 5 days (BASF, 1983b).

Mechanism of Degradation

Several bacteria strains capable of growth with NTA were isolated from wastewater, soil and sediment. An aerobic *Chelatobacter* strain a monooxygenase is responsible for the initial oxidation, leading to iminodiacetate (IDA) and glyoxylate. IDA is subsequently oxidized to glycine and glyoxylate by a membrane-bound dehydrogenase. Denitrifying bacteria contain a dehydrogenase/nitrate reductase complex, catalyzing the formation of IDA, glyoxylate and nitrite from NTA and nitrate (Egli, 1992, 1994).

The reaction pathway supports the result of the standard tests that NTA is completely mineralized after primary degradation. Accumulation of a stable metabolite is not expected.

Table 3.1: Results of laboratory biodegradation tests

Type	Method	Duration [d]	Inoculum ¹	Na ₃ NTA conc. [mg/l]	Degradation	Lag phase [d]	Reference
Modified OECD Screening Test	OECD 301 E	14	River water	70	100%	5	BASF (1983b)
Modified OECD Screening Test	OECD 301 E	14	Industrial WWTP effluent	70	100%	5-11	BASF (1983b)
Modified OECD Screening Test	OECD 301 E	7	Adapted AS	70	100%	1	BASF (1983c)
Modified OECD Screening Test	OECD 301 E	12	Adapted AS	140	75-90%	2-5	BASF (1983c)
Sturm Test	CO ₂ evol.	9	Effluent from standtest	10 / 20	100%	-	BASF (1983d)
Manometric Respirometry Test	OECD 301 F	28	Industrial AS	250-360	92%	16	Strotmann et al. (1995)
Combined CO ₂ /DOC Test	other	28	Industrial AS	140	>95% DOC 91% CO ₂	2 (DOC) 5 (CO ₂)	Strotmann et al. (1995)
Modified Zahn-Wellens Test	OECD 302 B	28	Industrial AS	1400	96%	7	BASF (1983a)
Die-away Test	other	23	Municipal AS	210	100%	14	Takahashi et al. (1997)

¹) AS: activated sludge

Influence of heavy metals on biodegradation

It was demonstrated by several investigations that biodegradation of NTA is influenced by the metal speciation.

Bench scale batch activated sludge experiments were conducted with various NTA-metal complexes with initial concentrations (as H₃NTA) between 8 and 16 mg/l (Shannon et al., 1978). The inoculum was activated sludge from a treatment plant, the test was run at 5°C. Biodegradation could be approximated by a first-order reaction. The degradation rates are presented in the table below. Oxygen uptake values observed during the experiments indicated that, with the exception of mercury, there were no inhibitory effects by the metals.

Bolton et al. (1996) used the NTA-degrading bacterium *Chelatobacter heintzii* in batch experiments with 5.23 μM (=1.3 mg/l Na₃NTA) complex solutions. Biodegradation followed first-order kinetics. Glucose degradation experiments in the presence of NTA-metal complexes revealed that metal toxicity was not a factor limiting NTA degradation. To test the hypothesis that the rate of NTA degradation would decrease as the complex stability constant increases, metals with a range of NTA stability constants were chosen. Calculations of the aqueous speciation using the computer program MINTEQ demonstrate that the added complexes were the dominant species in all experimental solutions. No relationship was found between the magnitude of the thermodynamical stability constant for the complexes and the first-order rate constant. E.g. Fe(III) which forms the thermodynamical most stable complex is rapidly degraded. Instead of this, a correlation between degradation constant and the liability (i.e. the reaction rate of complex dissociation) was found. The authors assume that the Mg-complex is the exclusive substrate for NTA degradation, its formation is related to the relative rates of HNTA²⁻ formation from the metal complex dissociation.

Table 3.2: Influence of metal ions on biodegradation

NTA complex with	Degradation constant (h ⁻¹)		Stability constant logK _{st}	Dissociation constant [M ⁻¹ s ⁻¹]
	Shannon et al. (1978)	Bolton et al. (1996)	Bolton et al. (1996)	Bolton et al. (1996)
uncomplexed		0.672	0	
Pb ²⁺	0.072			4.4·10 ⁶
Ca ²⁺	0.067			
Co ²⁺		0.260	11.7	0.8-8·10 ⁵
Fe ³⁺	0.066	0.209	17.8	
Zn ²⁺	0.051	0.190	11.9	5.1·10 ⁵
Al ³⁺	0.057	0.124	13.7	
Cr ³⁺	0.040			
Cu ²⁺	0.025	0.114	14.2	1.1·10 ⁵
Cd ²⁺	0.008			2.1·10 ⁵
Ni ²⁺	0.005	0.063	12.8	7.5
Hg ²⁺	0			

Madsen & Alexander (1985) studied the degradation using sewage organisms taken from raw sewage of a municipal treatment plant. Ca-NTA was mineralized at concentrations of 1, 10, and 100 µg/l. As the concentration of Ca-NTA increased, the rate of breakdown increased. No mineralization of Al, Mg, H, or Fe-NTA was detected with the same concentrations after an incubation of 585 h.

The available investigations demonstrate that results received from tests with uncomplexed NTA resp. with Ca- or Mg-complexes should be interpreted with care. Both in waste water and the hydrosphere, heavy metal ions are present which may inhibit degradation. For the environmental exposure assessment, the degradation properties of the metal complexes instead of the uncomplexed agent have to be taken into account.

There are contradicting results about the degradable metal species. E.g. according to Bolton et al. (1996) the uncomplexed NTA is most rapidly degraded, Madsen & Alexander (1985) found that only the Ca-complex is mineralized. The contradicting results may be caused by specific degradation mechanisms in individual inoculi. There is an agreement that complexes with several heavy metals inhibit NTA degradation. Heavy metals are widely spread, considerable fractions of NTA may be bound to these metals thus influencing the agent's removal in treatment plants and in environmental compartments.

However, non-degradable complex species can be transformed into degradable compounds by metal exchange reactions. As in sewage and the hydrosphere always a mixture of complexes is present, a prediction of the degradation rates from standard laboratory tests is not possible.

Elimination in treatment plants

An extended study was conducted in the Netherlands using a pilot plant of some 3000 population equivalents capacity. The sewage (municipal with a high industrial fraction) was spiked with H₃NTA up to 40 mg/l in 3 steps. The observed adaption time was 0 – 9 days dependent on the concentrations. With a continuous NTA load, removal rates of 93.0 – 98.9% (average 96.2%) were observed after adaption. The removal decreased when the plant was overloaded with organic waste or when the temperature fell below 7°C (Pöpel et al., 1984).

In 1984, NTA was detected in influent and effluent of 2 German municipal biological treatment plants. The concentrations were in the range of 196 – 562 µg/l in the influent and 6.6 – 23.1 µg/l in the effluent, the calculated removal is 93.5% – 98.2% (average 96.2%). The measurements were carried out in winter with effluent temperatures in the range of 11 – 13°C. The hydraulic retention time was 5 – 10 h. For a further plant a removal of 90% is reported (Hansen, 1986).

In a Swiss municipal plant, influent concentrations of 300 – 1500 µg/l (diurnal variation) were detected. In both seasons, NTA was degraded to 97%. The average sludge age was 3.6 d in winter and 4.8 d in summer. The influent concentrations increased by a factor of 4 between 1984 and 1987, whereas the effluent concentrations rose only by a factor of 1.5 (Alder et al., 1990).

For a Canadian municipal activated sludge plant, a removal of >95% in summer and less than 50% in winter is reported. The winter temperature in the plant is not reported, in the receiving creek the range was 0.5 – 3°C (Shannon et al., 1974).

The influence of temperature on the removal rate was investigated by Stephenson et al. (1983b) using activated sludge pilot plants. With 15 mg/l H₃NTA and typically occurring heavy metal concentrations, the removal rate (98%) was essentially unchanged when the temperature decreased from 17.5 to 10°C, further temperature reduction to 6°C caused a decrease in removal to 85%. With higher heavy metal concentrations (typically for industrial sewage), the removal was 95.2% (17.5 °C), 92.9% (10°C), and 79% (6°C).

In a pilot trickling filter plant, NTA was degraded incompletely. The removal of 20 mg/l in the influent was 70 - 80% above 10°C and 60 - 70% below 10°C in winter. With 40 mg/l in the influent, a removal of 67 – 82% above 10°C and <50 – 67% was observed. In a pilot oxidation ditch, the removal was <75% below 10°C (Heide, 1983).

The anaerobic degradation in sewage sludge was studied by Stephenson et al. (1983a). H₃NTA was added in concentrations of 10 – 30 mg/l to four laboratory scale anaerobic digesters treating mixed primary sludge and incubated at 35°C. After periods of adaption between 4 and 16 days, NTA was removed within some days. When NTA addition was interrupted, a memory effect was observed over periods up to 30 days.

The available studies reveal that NTA is removed in municipal treatment plants with rates generally above 95% under normal operation conditions. Contradicting results were obtained for measurements in the winter season: while in several cases no difference between summer and winter was observed, other studies show considerable decrease at low temperatures. Removal is disturbed when the temperature falls below a threshold of about 7°C. For the exposure calculations in this assessment, a removal of 95% is used, having in mind that in harsh winters the NTA releases can be increased.

Biodegradation in natural waters

NTA degradation was studied in a Canadian creek receiving municipal sewage from a biological treatment plant. The study was designed to determine the influence of temperature under summer and winter extremes. NTA was monitored at 6 sampling stations downstream. During the summer period, the NTA concentration was consistently less than the detection limit of 10 µg/l. In winter when the stream temperatures ranged from 0.5 to 3°C and NTA removal through the treatment plant was less than 50%, the downstream concentrations averaged to 106 µg/l. Considering the flow conditions, there was still evidence of biodegradation although it was considerably reduced. To determine the reaction rates, laboratory studies were carried out: in a 50 l tank containing creek water and a 10 cm sediment layer, primary degradation of 200 - 250 µg/l H₃NTA was determined at temperatures between 2 and 18°C. The degradation could be approximated by a first-order reaction with a rate constant ranging from 0.036 h⁻¹ (t_{1/2} = 19 h) at 18°C to 0.006 h⁻¹ (t_{1/2} = 115 h) at 2°C (Shannon et al., 1974).

Acclimation to and biodegradation of NTA was studied at trace concentrations in several river waters. Uniformly labelled ¹⁴C-Na₃NTA was added in various concentrations (1 – 1000 µg/l) at 14 or 24°C. In river waters not previously exposed to NTA, acclimation and degradation

were observed at the lowest concentration tested, which indicates that no threshold effect is expected. Degradation was preceded by a 5 – 10 day lag phase, and followed first-order kinetics after acclimation, the mineralization half-life ranged from 7 to 138 h at initial concentrations of 50 and 5 µg/l, respectively. In water samples where prior NTA exposure had already occurred no acclimation was required and degradation ($t_{1/2} = 7 - 17$ h) was less variable than in unexposed rivers (Larson & Davidson, 1982).

The effect of temperature on NTA degradation was investigated by Kari (1994). In the Swiss river Glatt receiving a high load of waste water, over a flow distance of 22 km about 90% of NTA was eliminated in summer and 65% in winter.

The biodegradation of NTA in the estuarine environment was examined by using a laboratory estuarine simulation (Hunter et al., 1986). Series of 5 reaction vessels were arranged in a stepped sequence, and a saline gradient (1.11 – 17.5 ‰) was achieved. The retention time for each vessel was between 5.6 and 8.3 h. Natural microbial populations from freshwater and marine sources were used as inoculum. Na₃NTA concentrations (0.9 – 1.02 µg/l) were removed to more than 95% in a salinity up to 8.77‰ after an acclimation time of 11 - 15d. with higher salinities, the removal decreased to minimum 48%. Na₃NTA concentrations of 6.95 mg/l (which is above the expected environmental level) were not removed in salinities above 20‰. The authors conclude that bacteria are unable to acclimate to NTA at moderate salinities.

The effects of salinity and DOC on the kinetics of biodegradation of NTA were studied in a Canadian river estuary with a prior history of NTA exposure (Larson & Ventullo, 1986). Water samples were collected in distances of 300 – 4600 m from the outfall of a primary treatment facility. U¹⁴C-NTA was added in a range of 10 – 100 µg/l, and ¹⁴CO₂, ¹⁴C activity in biomass, and ¹⁴C remaining in solution was measured. Degradation occurred immediately with no apparent lag phase, indicating that the microbial communities were adapted as a result of prior NTA exposure. There was no consistent effect of salinity (range 4 – 19 ‰) or DOC (range 2 – 12 mg/l) levels on NTA degradation rates. Degradation followed first-order kinetics, the estimated mineralization half-life was about 2 days.

The biodegradation of U¹⁴C-NTA at concentrations of 1 – 1000 µg/l in German estuarine water was determined by Hales & Ernst (1991). The final extent of degradation was lower at high salinity and low NTA concentrations. The shapes of the biodegradation curves was biphasial, degradation occurred in two stages in cases where microbial growth was required for complete degradation. From the initial phase, occurring under all conditions of temperature and salinity, half-life of 4 – 29 days at 12.2 °C were determined. The second stage was fitted by non-linear regression with the integrated Monod growth model: the 1000 µg/l concentration gave μ_{max} values in the range of 0.9 – 1.7 day⁻¹.

The available studies demonstrate that NTA is biodegraded in freshwaters with half-life in the range of several hours to some days, after an acclimation period in the range of days to weeks. Degradation is considerably influenced by temperature. For the following exposure calculations, a half-life of 5 days is used.

Contradictory results have been reported on its degradation in marine or estuary waters. Whereas Hunter et al. (1986) found that marine bacteria were unable to acclimate, rapid degradation ($t_{1/2}$ 2 d) was found for Canadian rivers with a prior history of NTA release (Hales & Ernst, 1991). Without acclimation increased half-lives up to several weeks are expected. The latter results are not considered in the assessment, as in the time frame of several days in estuaries dilution processes are predominating.

Biodegradation in sediments

Adaptation of bacterial activity for the primary degradation of NTA was studied using natural sediment samples and an NTA-degrading bacterium (*Pseudomonas sp.*). Sediment samples collected from a river loaded with persistent NTA levels degraded NTA with a half-life of about 1 d at a temperature of 30°C. The reaction rate with the pure bacteria strain associated with sand was approximately one order of magnitude slower. With sediment from a less contaminated control site a 5 day lag preceded an abrupt increase in NTA degradation (McFeters et al., 1990).

The only available study demonstrated a NTA degradation half-life of 1 d. However, the temperature (30°C) is far above typical environmental conditions, thus the real degradation rate is possibly lower. For the exposure calculations, for the aerobic sediment layer the same half-life (9 d) than for soils is used.

No studies with anaerobic sediments are available. NTA was found to be degradable in anaerobic sludge (Stephenson et al., 1983a) and with soil organisms under anaerobic conditions (Tabatabai & Bremmer, 1975; cited below). The reaction was slower than for the aerobic degradation. For degradation in the anaerobic sediment layer, a roughly estimated half-life of 20 d is used.

Biodegradation in soil

The primary degradation of NTA in 5 soils was studied by performing analyses for NTA and inorganic N. After 5 d incubation at 30°C, 12 - 45% of the Na₃NTA (initially 200 mg/kg) was recovered under aerobic and 20 - 65% under anaerobic conditions. After incubation under anaerobic conditions the NTA-nitrogen was in the form of NH₄⁺, while under aerobic conditions NO₃⁻ was formed (Tabatabai & Bremmer, 1975).

Mineralization of U¹⁴C-NTA was measured in batch test systems containing groundwater or groundwater plus subsurface soil material (GWSS slurries) at 20°C. In groundwater, with an initial concentration of 50 µg/l a half-life of 54 h was determined under aerobic conditions. About 80% of the radioactivity was evolved as ¹⁴CO₂, while 20% were incorporated in biomass. In the GWSS slurry (10 g soil + 10 ml groundwater), NTA (initially 5 µg/l) was degraded with half-lives of 155 h under anaerobic and 128 h under aerobic conditions (Larson, 1984).

Shimp et al. (1994) assessed the mineralization of U¹⁴C-NTA in soil and groundwater samples from a septic tank system plume. The assays were incubated at 20°C, mineralization of initially 25 – 100 ng NTA/l was measured as ¹⁴CO₂ evolution. In soil samples, activity was generally highest in the area immediately to the septic tank tile field, resulting in half-lives of < 3d. At 20 m downgradient from the field, there was little biodegradative activity (not quantified). Similar results were obtained with groundwater samples: nearest the tile field a half-life of 0.78 d was determined, while in 40 m distance the half-life decreased to 9 d.

Degradation of NTA was investigated in a water/soil system simulation the underground passage (Stumpf et al., 1996). Samples with a soil/water ratio of 1/0.7 were dosed with 50 mg/l H₃NTA, incubated for 10 weeks, and the remaining NTA measured by GC. In the Ah- and Go horizons, after a lag-phase of 5 days the substance degraded rapidly (t_{1/2} 1-2 d). In

an aquifer sampled at an infiltration area of a drinking water work no lag phase was observed, indicating pre-adaption. The half-life was about 5 d, after 15 d the test substance was completely degraded. In all samples NTA metabolites were not detected.

The available studies demonstrate that NTA is mineralized in soils, half-lives between 1 – 9 days were determined. For the exposure calculations, a half-life of 9 d is used as a worst case.

3.1.1.2.2 Photolytical degradation

The Fe(III)NTA complex was found to undergo photolysis when exposed to sunlight. In a 1 mM aqueous solution irradiated with sunlight in July ($t = 25 - 30^{\circ}\text{C}$), the concentration of NTA decreased rapidly over a period of 9 h. As reaction products, iminodiacetate (IDA), CH_2O and CO_2 were detected. IDA is very slowly degraded to glycine under the same test conditions. Complexes with other metals were irradiated for a week: while no significant change was found for Pb(II) and Cd(II), a marginally decrease for Cr(III) and a marked (70%) decrease for Cu(II) was observed (Stolzberg & Hume, 1975).

Svenson et al. (1989) determined the quantum yield for Fe(III)NTA photolysis to 0.0129. For the yearly maximum of a solar spectrum at 60°N , a half-life of 42.9 min was calculated. This value refers to the top millimetres of a water body at noon, because of factors like cloudiness, shadowing effects of vegetation, absorption and scattering of light by suspended solids etc. the actual environmental lifetime is certainly longer.

The available studies demonstrate that in surface waters photolysis can contribute to NTA degradation. As referred in section 3.1.2.3, in the thermodynamical equilibrium state there are only small fractions of the photolytically instable complex species. Biodegradation is the predominant degradation mechanism, thus photolysis is not considered in the exposure assessment.

Summary of degradation rates

The following degradation rates are used in the further exposure assessment:

Parameter	Degr. rate	Half-life
$k_{\text{bio}_{\text{water}}}$	0.14 d^{-1}	5 d
$k_{\text{bio}_{\text{sed}}}$ (aer)	0.077 d^{-1}	9 d
$k_{\text{bio}_{\text{sed}}}$ (anaer)	0.035 d^{-1}	20 d
$k_{\text{bio}_{\text{soil}}}$	0.077 d^{-1}	9 d
$k_{\text{deg}_{\text{air}}}$	0 d^{-1}	∞

3.1.1.3 Distribution

Because of the salt character of NTA no value for the vapour pressure is available, therefore Henry's law constant cannot be calculated from vapour pressure and water solubility. Volatilization from aqueous solution is not expected. For the exposure calculations, the lowest value accepted by EUSES ($4E-10 \text{ Pa}\cdot\text{m}^3/\text{mol}$) is used.

Due to the ionic structure under environmental relevant pH conditions, a relevant adsorption onto the organic fraction of soils or sediments is not expected. However, interaction with the mineral phase is possible.

The distribution of NTA between a marine surface sediment and a mineral medium (used for biodegradation tests) was examined by Bolton et al. (1993). After addition of $10 \mu\text{M}$ NTA, a mixture of complexes is formed. After 24 h incubation at a temperature of 4°C , a distribution coefficient **Kp of 1.6 l/kg** was determined. In the exposure calculations, this value is used for adsorption onto both sediments, suspended particles, and soils.

3.1.1.4 Bioaccumulation

The uptake of NTA and its complexes by a series of species was examined in detail by Lentz & Lidzba (1988). With a Na_3NTA concentration of $400 \mu\text{g/l}$, the following BCF values were obtained:

Table 3.3: BCF values for aquatic species

Species	BCF [l/kg]	Equilibrium after
<i>Fish (Brachidanio rerio)</i>	1 - 3	96 h
<i>Guppy (Lebistes reticulatis)</i>	male 1 - 2 female 6	72 - 96 h
<i>Goldfish (Carassius auratus)</i>	1 - 2	72 - 96 h
<i>Snail (Lymnaea stagnalis)</i>	8 ≥ 20	3 - 7 d $\leq 72 \text{ d}$
<i>Notonecta spec.</i>	2 - 4	48 h
Tubificidae	5 - 10	5 d
Frog larvae (<i>Rana temporaria</i>)	5 - 10	96 h
Frog (<i>Rana temporaria</i>)	< 1	
Crayfish (<i>Procambarus</i>)	1	4 h

In the same study, *Brachidanio rerio* was exposed to different NTA complexes. BCF values of 1.9, 3.4, and 2.8 were obtained for Cu-NTA, Cd-NTA, and FeNTA, respectively.

The available study demonstrates that only a low accumulation of NTA occurs in the hydrosphere. For the exposure calculations, a **BCF** value of **3 l/kg** is chosen.

3.1.2 Aquatic compartment

3.1.2.1 Estimation of PEC_{local} during production

In the *Technical Guidance Documents for New and Existing Substances*, release factors into the raw sewage of 0.3% for production (TGD, appendix I, table A1.1) and 0.3% for formulation are proposed as default values.

In this section the exposure is calculated from specific data for 4 production sites: from the emission and production volumes, release factors from 0.4 ppm to 0.85 % are calculated. In these factors waste water purification is included.

For calculating the C_{local,aqua}, the dilution of the waste water in the river is considered according to

$$C_{\text{local,water}} = C_{\text{local,eff}} \cdot D \quad \text{with } D = Q_{\text{ww}} / Q_{\text{river}}$$

C_{local,eff}: concentration in wwtp effluent

D: dilution factor

Q_{ww}: sewage flow

Q_{river}: river flow (10%ile value preferred)

$$\text{PEC}_{\text{regional}} = 4.2 \text{ } \mu\text{g/l (cf. 3.1.2.4)}$$

In the following table, the estimated concentrations, releases into the environment, and the underlying specific data (as far as available) are summarized:

Table 3.4: Exposure from Na₃NTA production

Site	Site-specific data	Defaults	Ceff [mg/l]	Clocal _{aqua} [µg/l]	PEClocal _{aqua} [µg/l]	Release
A	only import	-	0	0	-	0
B	Effluent conc.; release period 20 times/a for 24 h, sewage flow	dilution 1:10	No wwtp	< 0.93	< 5.1	<370 g/a
C	Effluent conc.; sewage and river flow	-	0.054	1.4	5.6	7.6 t/a
D	Daily release, sewage flow		No wwtp	< 450	< 450	10.8 t/a
E	yearly release volume, production period; sewage and river flow	-	1.1	9.4	14	5.7 t/a

Remarks:

Site D: The concentrations in the receiving estuary are modeled, resulting in maximum values in the range of 0.25 – 0.45 mg/l.

The total release at the production sites into surface waters is 24.1 t/a Na₃NTA.

3.1.2.2 Estimation of PEClocal during Formulation and Use

3.1.2.2.1 Textile Cleaning

NTA is an effective substitute for phosphates. It prevents calcium and magnesium ions from forming sparingly soluble salts with orthophosphate, pyrophosphate, carbonate, silicate and other inorganic anions as well as anionic surfactants and fatty acids (BASF, 2001).

In 2000, 973 t Na₃NTA were used for textile cleaning, in both household and industrial applications. As no breakdown between household and industrial use is available, as a worst case approach the exposure calculation is based on industrial use. Furthermore, it is assumed that releases occur only into waste water. All other parameters are taken from the Technical Guidance Documents. The 10 % rule was applied due to the assumed wide and equal distribution of the sites over the EU (cf. table 2.1).

Table 3.5: PEC calculation for textile cleaning

	Formulation	Use
Total use volume	973 t/a	
Continental use	876 t/a	
Regional use	97 t/a	
Fraction of local main source	1	0.4
Number of emission days	300 d/a	194 d/a
Fraction released to waste water	0.02	1
Release into untreated waste water	6.5 kg/d	200 kg/d
Release into hydrosphere (removal 95%)	0.32 kg/d	10 kg/d
Ceffluent	160 µg/l	5000 µg/l
Clocal	16 µg/l	500 µg/l
PEC_{local} (PEC _{regional} = 4.2 µg/l)	20 µg/l	500 µg/l

3.1.2.2.2 Cleaning agents

NTA help prevent water-based formulations, especially neutral and alkaline liquid cleaners, from becoming cloudy or precipitating.

In 2000, 17905 t Na₃NTA were used in cleaning agents for industrial applications. It is assumed that releases occur only into waste water. All other parameters are taken from the Technical Guidance Documents.

Table 3.6: PEC calculation for cleaning agents

	Formulation	Use
Total use volume	17905 t/a	
Continental use	16115 t/a	
Regional use	1790 t/a	

	Formulation	Use
Fraction of local main source	1	0.002
Number of emission days	300	200
Fraction released to waste water	0.003	1
Release into untreated waste water	17.9 kg/d	17.9 kg/d
Release into hydrosphere (removal 95%)	0.895 kg/d	0.895 kg/d
Ceffluent	450 µg/l	450 µg/l
Clocal	45 µg/l	45 µg/l
PEClocal (PECregional = 4.2 µg/l)	49 µg/l	49 µg/l

3.1.2.2.3 Sediments

No monitoring data for sediments are available. The Na₃NTA concentration could be modelled using the equilibrium partitioning method. As also no effect tests are available, a risk assessment for sediments would lead to identical PEC/PNEC ratios like for the aquatic compartment.

Because of the low partitioning coefficients, no accumulation in sediments is expected. Thus an assessment of this sub-compartment is not necessary.

3.1.2.2.4 Monitoring

In contrast to most publications about environmental fate of ecotoxicology where NTA amounts or concentrations are referred as sodium salt, monitoring data are generally referred as H₃NTA. In this section, the original data are cited. In order to receive figures comparable to the other sections of this report, the H₃NTA figures have to be multiplied with a factor of 1.35 to receive the Na₃NTA equivalents.

Monitoring in surface waters

NTA is continuously monitored in German surface waters (LAWA, 2000). In 1997/98, the substance was sampled at 84 locations at 51 rivers and creeks, mostly with 13 samples per year at each location. From totally 2283 measurements, the highest detected concentration was 100 µg/l H₃NTA (= 135 µg/l Na₃NTA). In the following table, the results are sorted in concentration ranges, the 90%ile values are considered:

Table 3.7: 90%ile values of H₃NTA concentrations in German surface waters (LAWA, 2000)

90%ile concentration [µg/l]	Number of sites
0 - 1	2
1 – 10	65
10 - 100	17
total	84

An survey of concentrations of NTA in UK rivers and at sewage treatment works was reported by FWR (1992). Sampling was carried out in April and May 1992 at 25 river sites and 10 sewage treatment works. NTA concentrations in river water ranged from less than the detection limit of 2 µg/l to 43 µg/l with a log normal distribution. In Class 3 rivers (NWC or Scottish Classifications, i.e., highly polluted rivers) the mean concentration was 16 µg/l and the median concentration was 10 µg/l. There were no identifiable differences between the means for the Class 1 or Class 2 rivers for which both the overall mean and the medians were less than 2 µg/l.

The following data were reported from an environmental survey in Austria: NTA was not detectable (detection limit: 2 µg/l) in 78 of 85 sampling places (number of samples for each location: 6). In six sample places, a maximum value between 6 and 10 µg/l was measured. In one case (sample from a channel near Vienna) the medium and highest values were 42 and 231 µg/l, respectively (FEA, 1996).

During a monitoring campaign in 10 Swiss rivers performed in 1990, the yearly averaged NTA concentrations were in the range of 0.8 – 10 µg/l (Giger et al., 1991). Measurements in 5 lakes near drinking water works performed in 1993 resulted in a maximum concentration of 0.5 µg/l (IAWR, 1993).

Monitoring in waste water

In the following table, an overview about measurements in waste water is presented. Generally there is no information about the origin of the NTA.

Table 3.8: NTA concentrations in in- and outflow streams from sewage plants

Location	Year	I/O	Conc. NTA [$\mu\text{g/l}$]	Reference
Zurich, Glatt	winter 1984	Influent	40-380	Alder et al. (1990)
		Effluent	3-30	
	winter 1987	Influent	330-1490	
		Effluent	5-50	
Hessen	1987	Influent	100-300	Kröber & Häckl (1989)
		Effluent	<2-23	
Bielefeld-Heepen	1987	Influent	64-68	Lahl & Burbaum (1988)
		Effluent	8-16	
UK (10 plants)	1992	effluent	< 2 – 740	FWR (1992)

Comparing the available monitoring data with the calculated PEC's (cf. 3.1.2) it can be seen that there are mostly in the same order of magnitude. The calculated PEC for the use in textile cleaning (cf. table 3.5), which is based on a worst case approach, is clearly above the measured data.

3.1.2.3 NTA metal complexes in the hydrosphere

In natural waters, both natural and anthropogenic metals are present, which are able to form complexes with NTA. The environmental risk assessment is confronted with some problems (e.g. photolysis of complexes, remobilisation of sediment-bound metals, effects of heavy metals) which are related to the speciation under environmental conditions. Therefore the main features of the NTA complex chemistry have to be elaborated.

3.1.2.3.1 Stability of NTA complexes (Ringbom & Wänninen, 1979)

The most important property of NTA is to form complexes (usually 1:1-complexes) with multivalent metal ions. The stability of these complexes is usually described by the mass action law:

$$K_{MeZ} = \frac{[MeZ^{(m-n)-}]}{[Me^{n+}] \cdot [Z^{m-}]}$$

with	$[MeZ^{(m-n)-}]$	the concentration of the metal complex
	$[Me^{n+}]$	the concentration of the metal ion
	$[Z^{m-}]$	the concentration of the NTA ³⁻ anion (active complexing species)
	K_{MeZ}	the stability constant of the metal complex

The stability of the complexes of the N(CH₂COO⁻)₃ anion with a polyvalent metal ion is described by the stability constants listed in Table 3.9 (K₁ for 1 to 1 complexes and K₂ for 2 to 1 complexes). As a result of polarisation of the OH bond in the chelate, the 1 to 1 complexes behave like weak acids and also dissociate. This effect is expressed by the dissociation constant K_d.

Table 3.9: Different stabilities of NTA chelates formed with various metal ions (Martell & Smith, 1974)

Metal ion	log K ₁	log K ₂	pK _d
Al ³⁺	11.4		5.09
Ca ²⁺	6.39	8.76	
Cd ²⁺	9.78	14.39	11.25
Co ²⁺	10.38	14.33	10.80
Cu ²⁺	12.94	17.42	9.14
Fe ²⁺	8.33	12.80	10.60
Fe ³⁺	15.90	24.30	4.1 (7.8 ^a)
Hg ²⁺	14.60		
Mg ²⁺	5.47		
Mn ²⁺	7.46	10.94	
Ni ²⁺	11.50	16.32	10.86
Pb ²⁺	11.34		
Zn ²⁺	10.66	14.24	10.06

^a : Reacts as dibasic acid, ionic strength (25 °C) 0.1 M.

The distribution of the specific metal complexes in the hydrosphere cannot be derived directly from the mass action law, because of the following reasons:

- In aqueous solution, NTA can in principle occur as a neutral molecule or as ions with different charges. With increasing pH, ionisation increases and the formation of complexes is enhanced.
- Metals can form insoluble hydroxides (especially in alkaline medium), phosphates and carbonates, complexes with other ligands (e.g. humid substances) or can be adsorbed onto suspended solids, which decrease the concentration of free metal ions. Some of these reactions are also dependent on pH.
- Both effects are accounted by the conditional complex-formation constant. These constants pass for all metal complexes through a maximum as a function of pH value.

An example is iron that according to Table 3.9 forms the most stable Fe(III)NTA complex ($\log K_1 = 15.9$). In addition iron is the most frequent transition metal in river water. This would suggest that the major product formed under environmental conditions is the Fe(III)NTA complex. Nevertheless, studies on the NTA speciation in surface waters (see below) reveal that no significant amounts of Fe(III)NTA are present in the thermodynamic equilibrium state, as insoluble $\text{Fe}(\text{OH})_3$ and $\text{Fe}(\text{O})\text{OH}$ are formed which are adsorbed or form colloids.

Taking into account the different molecular weights of $\text{N}(\text{CH}_2\text{COONa})_3$ (MW.: 257 g/mol) and the metal ions, and on the assumption that a 1 to 1 complex is formed, 1 mg Na_3NTA hypothetically can bind the following amounts of metal ions in the optimum pH range (Table 3.10):

Table 3.10: Complexing capacity of Na_3NTA

Metal	MW [g/mol]	Me. bound by 1 mg Na_3NTA [mg]
Transition metal		
Cu^{2+}	63.5	0.25
Zn^{2+}	65.4	0.25
Ni^{2+}	58.7	0.23
Fe^{3+}	55.8	0.22
Alkaline earth metal		
Ca^{2+}	40.1	0.16
Mg^{2+}	24.3	0.09

3.1.2.3.2 Exchange reactions of metal complexes

When metal complexes come into contact with other metals, metal exchange reactions occur. Complexes being wasted from any technical process reach a treatment plant and are mixed with other waste waters, leading to a change in the speciation. The same processes occur when the effluent is released into surface waters with a different metal composition. The mechanism of metal exchange reactions is dissociation of a metal complex with subsequent binding of another metal ion. The rate of the exchange reaction is limited by the rate of the dissociation of the mother complex. The reaction rates vary in a large range. For NTA, reaction rates in the order of hours for Ni and seconds for Zn are quoted (Bolton et al., 1996).

3.1.2.3.3 Effect on heavy metals in treatment plants

Raw sewages always contain a more or less high amount of heavy metals. In general heavy metals are strongly adsorbed onto sewage sludge thus being removed from the water phase. By complexation with agents like NTA the metals are kept in the soluble phase, when the chelator is biologically degraded the metal ions are set free and adsorbed. When the chelator is not completely degraded, in the effluent increased metal concentrations can occur.

The influence of NTA on heavy metal concentrations in a Swiss municipal treatment plant effluent was studied by Alder et al. (1990) by measurements of NTA and some heavy metals in the effluent. With a normal load ($20 \pm 15 \mu\text{g/l}$ H_3NTA in the effluent), remobilization was found to be neglectable compared to the normal load. During a shock loading (up to $2000 \mu\text{g/l}$ in the effluent), Zn and Pb were remobilized from sludge, and their effluent concentrations increased by 200 and 50%, respectively. The concentration of copper did not increase.

Similar results were obtained by Pöpel et al. (1984). The effluent concentrations of Ni, Pb, Zn were increased during shock loading (40 mg/l H_3NTA in the influent) when NTA degradation was disturbed. Under normal operation conditions the metal levels were not increased.

It can be concluded that increased heavy metal releases into the hydrosphere can occur with high NTA loads or when NTA degradation is disturbed. Simultaneously, the sludge deloaded is leading to a decreased contamination of agricultural soil when sludge is applied as fertilizer.

3.1.2.3.4 Speciation of NTA metal complexes in the hydrosphere

So far, no analytical methods are available to differentiate the individual metal complex species. During sample preparation, the metal complexes are destroyed, the sum of all species is determined, generally being expressed as amount of the uncomplexed agent.

Possibilities to approximate the metal complex speciation are model calculations, which describe the state of thermodynamical equilibrium. With complex models, the chemical interactions and the competition between trace metals and different constituents in natural waters can be investigated. Naturally occurring chelators like humic acids can compete with the anthropogenic complexing agent, thus their inclusion into the calculation model is absolutely necessary. These models have to consider a large series of components, e.g.

- the major cations Ca, Mg, K, Na
- the trace metals Pb, Cu, Ni, Zn, Cd, Co, Hg, Mn, Fe
- the inorganic ligands CO₃, SO₄, Cl, F, Br, NH₃, PO₄, OH
- the adsorbent SiO₂ to represent suspended solids.

A speciation calculation using the model MULTI was conducted by Hennes & Eberle (1984), which studied the influence of H₃NTA (10 µg/l – 10 mg/l) on metal concentrations typically for the river Rhine. With 100 µg/l H₃NTA, Cu, Pb, and Ni are predominantly complexed, while above 500 µg/l Zn and Cd are strongly bound. With 1 mg/l NTA, the fraction of CaNTA increases to 85%. In the table below, the results for an NTA concentration of 100 µg/l are presented.

Different results were obtained by a speciation calculation predicted for the Swiss river Glatt. With a concentration of 2 µg/l, NTA is predominantly complexed with Ca, and only a small portion would be associated with heavy metals. The authors explain the discrepancy to the study of Hennes & Eberle (1984) by inclusion of natural ligands for Cu and Zn, which compete successfully with NTA for the metal ions (Bucheli-Witschel & Egli, 2001).

Table 3.11: Calculated Speciation of NTA in surface waters. Fractions [Me-NTA]/[NTA] in %

Metal	Hennes & Eberle (1984)	Bucheli-Witschel & Egli (2001)
Ca	25	95
Mg		3
Ni	25	
Zn	30	<1
Cu	15	

Attention has to be given to the fact that the modelled results are based on highly variable parameters like concentration of the complexing agent, metal composition of surface water etc. Furthermore, only the thermodynamical equilibrium state is modelled, degradation reactions are not considered. At the thermodynamic equilibrium, the complex formation is selective. The most preferred metals being complexed are those with the highest conditional complex-forming constants. With increasing concentrations, other metal ions are complexed successively. In German and Dutch rivers, heavy metal concentrations in the range of 10-20 µmol/l (predominantly Fe and Mn) are detected. The PECs for NTA are always lower, thus in the hydrosphere NTA is always completely complexed.

In seawater, complexation of heavy metals is not important because of the low NTA concentrations and the high Ca- and Mg levels (Bernhardt, 1991).

3.1.2.3.5 Influence on the partitioning of heavy metals in sediments and water

In the hydrosphere, presence of complexing agents can cause an increase in soluble levels of heavy metals. There is a tendency to remobilize the metals from highly loaded sediments which remain from high metal emissions in the past. On the other hand, adsorption of recently emitted heavy metals onto sediments and suspended solids could be prevented.

In a series of laboratory studies using sediment slurries remobilization of adsorbed metals was observed. Dependent on the nature of sediments and the water phase, increasing metal concentrations were observed with NTA concentrations generally above 1 mg/l. The lowest concentrations were found with sediment from a sea harbour; remobilization of Ni and Cu occurred with 0.1 mg/l and Zn with > 0.1 mg/l H₃NTA. In hard waters the remobilization degree is substantially lower because of the competition of Ca (Bernhardt, 1991).

In real surface waters, the distribution of metals is not only determined by the complex formation properties, but also by a series of physical, chemical and microbiological interactions. The results of sediment slurry experiments describe the maximum remobilization capacity of a complexing agent, i.e. a worst case situation.

An extended study on heavy metal remobilization by NTA closer to natural conditions was performed by Lorenz (1997). Bottom-layered sediment bodies (8 natural lake and river sediments) were overflowed by artificial river water containing 400 resp. 1,600 µg H₃NTA/l (i.e. 2.13 resp. 8.51 µmol/l), and the increase of heavy metals in the water phase was detected. During the test time (up to 42 days) NTA both aerobic and anaerobic degradation was observed with half-lives of about 6 and 20 days, respectively. Typical concentration patterns specific to each element were found, which generally were independent of the choice of sediment. At aerobic water phase (8 mg O₂/l) a 2 mm deep oxic capping sediment layer developed out of which an NTA dependent remobilization can occur. Under "worst case" conditions (highly polluted sediment from river Mulde, 2000 µg/l Na₂HNTA) mainly Zn was temporarily remobilized (1.5 µmol/l), while a significantly lower, temporary conversion for Pb (up to 0.3 µmol/l) was observed. The metal concentrations in water went through a well marked maximum after 3 – 5 days, i.e. the remobilization process is reversible. No remobilization of Fe, Mn, Cd, Cu, Ni, P or S was detected.

Anaerobic conditions were received by overflowing the sediment with oxygen-free water. No substantial remobilization of the toxic metals Zn, Pb, Cd, Cu and Ni was observed. This is explained by formation of metal sulphides with an extreme low solubility. Thus, in nature even highly loaded sediments cannot release heavy metals as long as they are buried in deeper sediment layers. However, if anoxic sediment layers come into contact with oxygen (which occurs by whirling up during high water flows or by shipping traffic), sulphide is oxidized to sulfate and the metals are again available for remobilization.

The author concludes that the extent of remobilization is not primarily determined by the complex formation constants. Higher influence has the sediment load and the form of binding of the metals (Lorenz, 1997).

Within the framework of the NTA research program two approaches were selected in Germany. In the first experiment, the diffusion of heavy metals from an artificial sediment was determined in the presence and absence of free NTA in laboratory experiments (Donnert et al., 1991). From resting (i.e., none suspended) kaolinite as the model sediment the addition of 850 µg/l of (free) H₃NTA duplicated the Zinc concentration in water. The process was

developing very slowly and did not attain the equilibrium state after several hundred hrs. A mathematical model based on the Langmuir sorption relationship was developed from this experiment for the description of the interaction between heavy metals, clay mineral layer and the water phase. The calculation was carried out for zinc according to the conditions of river Rhine, i.e., 250 hrs contact time and the corresponding geometrical as well as chemical parameters like the initial zinc concentration of 230 $\mu\text{g/l}$ in the water. The calculation predicts that approximately 10% (25 $\mu\text{g/l}$ of Zn) of the initial zinc concentration can just be significantly remobilized by 200 $\mu\text{g/l}$ of H_3NTA not yet bound on heavy metals.

In the second experiment, the concentrations of dissolved copper, nickel, and zinc were determined in a 1000 m flume filled with river sediment over which water with or without the addition of uncomplexed NTA flowed at a slow rate (Bernhardt, 1991). A definite remobilization of the widely distributed and easily remobilizable zinc began at a concentration of 50 $\mu\text{g/l}$ of free NTA not yet bound on heavy metals. Other heavy metals, such as copper and nickel, were only remobilized at clearly higher NTA concentrations. In this flume experiment, an NTA end concentration of less than 20 $\mu\text{g/l}$ of NTA was always obtained because of biodegradation on a flow section of approximately 900 m above a temperature of 10 °C and independent of the initial NTA concentration (50 – 500 $\mu\text{g/l}$ of NTA).

For the evaluation of the remobilization process, the following topics need to be considered:

- NTA always occurs as metal complex in the hydrosphere. In German rivers, heavy metal concentrations in the order of range of 10-20 $\mu\text{mol/l}$ (predominantly Fe and Mn) are detected. The stoichiometric Na_3NTA equivalent is 2.6 - 5.1 mg/l. All PECs are below this range. Therefore, all NTA is bound onto metals, and there is no free NTA available to remobilize metals from sediments. Only metal exchange reactions may occur.
- In remobilization tests with freshly adsorbed heavy metals, some metals begin to remobilize when the NTA concentration exceeds 100 $\mu\text{g/l}$.
- Old highly loaded sediments remain in deeper (anoxic) sediment layers. Remobilization from the deeper layers is limited by formation of nearly insoluble metal sulphides. Only if the sediments are whirled up during high water flows, a significant increase of heavy metal abundance in the water phase can occur.
- The more time has gone since the reduction of high metal emissions, the lower is the probability that these old sediments come into contact with the river water being available for metal remobilization.
- It is not possible to give a single value for an NTA concentration at which no effects on metal remobilization occurs. Because of the complexity of the NTA-metal interactions (dependent on metal concentrations, pH, nature of the sediment, concentration of organics etc.), it is not possible to come to a general rule for effects which is applicable to each river system. For individual surface waters, model calculations can be performed to receive a rough estimation.

It can be concluded that significant remobilization processes can only occur in extreme cases, i.e. when high NTA amounts are released. This leads to an increase of metals with high

conditional complex- formation constants. In this case they would be completely complexed with NTA. Simultaneously, the sediments are deloaded.

Further investigations dealing with the mobility of heavy metals during bank filtration are cited in section 4.1.1.4.

3.1.2.4 Regional exposure

For the regional exposure assessment it is assumed that the total European Na₃NTA consumption volume (26642 t in 2000) is released into the waste water. In accordance to the TGD, 10% of the European releases are taken for the regional and 90% for the continental scenario. In the following table, the release amounts and the resulting PECs of the EUSES calculation are presented (cf. Appendix A I):

Table 3.12: Calculation of PEC_{regional}

Parameter	regional	contin.
Release into waste water [t/a]	2664	23978
Release into wwtps (70%) [t/a]	1865	16785
Release via wwtp effluents [t/a]	93	839
Direct release into hydrosphere (30%) [t/a]	799	7193
Total release into hydrosphere [t/a]	892	8032
PEC _{water} [µg/l]	4.2	0.48
PEC _{air} [µg/m ³]	9.4E-16	1.1E-16
PEC _{agric.soil} [µg/kg dw]	6.9E-10	7.9E-11
PEC _{ind.soil} [µg/kg dw]	2.6E-9	3.0E-10
PEC _{nat.soil} [µg/kg dw]	2.6E-9	3.0E-10
PEC _{sediment} [µg/kg dw]	4.2	0.48

3.1.3 Atmosphere

No relevant releases into the atmosphere are expected.

3.1.4 Terrestrial compartment

No relevant releases into soils are expected.

3.1.5 Non compartment specific exposure relevant to the food chain

From the ionic structure of sodium NTA it can be concluded that a significant accumulation of these substances in the biota is not to be expected.

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1 Aquatic compartment (incl. sediment)

A result of the exposure assessment was that in the environment over-stoichiometric amounts of metal ions are present, thus NTA is always complexed with metal ions. In section 3.1.2.3, it is elaborated that always a mixture of different metal complex species occurs in surface waters. Similar reactions take place in toxicity test media, where metal ions are complexed when uncomplexed NTA is added. For the interpretation of the test results the complex speciation has to be considered.

In the effect tests, either H₃NTA or the sodium salt were used as test substance. In order to present comparable results, all effect values are referred as Na₃NTA.

3.2.1.1 Toxicity to fish

There is a large database on the toxicity of NTA on fish, an overview of the results considered to be valid is presented in tables 3.13 and 3.14.

NTA is a readily degradable substance, and in some test media NTA is possibly not stable throughout the test period. E.g. Macek & Sturm (1973) measured as an average 173 mg/l Na₃NTA in a medium with a nominal concentration of 200 mg/l, and 3.4 mg/l at nominal 5.6 mg/l. Therefore, only studies conducted with analytical monitoring are considered in this section.

Static and flow-through tests using the bluegill sunfish (*Lepomis macrochirus*) were conducted both in soft (60 mg/l CaCO₃) and hard water (170 mg/l CaCO₃). Monitoring showed essentially no loss of NTA throughout the test, which was not unexpected as the media were either sterile or were made from distilled water. The LC50 values in the static test system were 487 mg/l in hard water and 252 mg/l in soft water, while under flow-through conditions the values were 476 mg/l and 278 mg/l, respectively. Microscopic examinations of the fish gill tissues revealed slight damage consisting of the loss of lamellar interdigitation, the beginning of this effect was observed at 155 mg/l in the hard water static test, 115 mg/l in

the soft water static test, 420 mg/l in the hard water flow-through and 370 mg/l in the soft water flow-through test (Weaver, 1970).

Table 3.13: Toxicity of Na₃NTA to fish in short-term tests

Species	Method	Test type	Test conditions			Exposure time	Effect conc. [mg/l]	Reference
			Temp [°C]	Hardness [mg/l CaCO ₃]	pH			
<i>Pimephales promelas</i>	APHA method	Flow-through	25 ± 1	ND	7.9 – 9.2	96 h	LC50 = 114	Arthur et al. (1974)
<i>Pimephales promelas</i>	APHA method	Flow-through	ND	35	ND	96 h	LC50 = 127	Macek & Sturm (1973)
<i>Lepomis macrochirus</i>	APHA method	Static	20 ± 1	60 170	ND	96 h	LC50 = 252 LC50 = 487	Weaver (1970)
<i>Lepomis macrochirus</i>	APHA method	Flow-through	20 ± 1	60 170	ND	96 h	LC50 = 278 LC50 = 476	Weaver (1970)
<i>Oncorhynchus mykiss</i>	APHA method	Flow-through	ND	35	ND	96 h	LC50 = 98	Macek & Sturm (1973)

(all concentrations related to Na₃NTA and based on analytical measurements)

Table 3.14: Toxicity of Na₃NTA to fish in long-term tests

Species	Method	Test type	Test conditions			Exposure time	Effect conc. [mg/l]	Reference
			Temp [°C]	Hardness [mg/l CaCO ₃]	pH			
<i>Lepomis macrochirus</i>	APHA method	Flow-through	19 ± 0.5	35	7.1	28 d	LC10 = 96 LC55 = 173	Macek & Sturm (1973)
<i>Pimephales promelas</i>	APHA method	Flow-through	19 ± 0.5	35	7.1	28 d	LC0 = 96 LC100 = 173	Macek & Sturm (1973)

<i>Carassius auratus</i>	Embryo-larval-test	Flow-through	18.2 – 25.8	50 200	7.9 – 8.1	8 d	LC1 = 28.5 LC50 = 240 LC1 = 30.1 LC50 = 243	Birge et al. (1979)
<i>Ictalurus punctatus</i>	Embryo-larval-test	Flow-through	25.9 – 29.6	50 200	7.9 – 8.1	9 d	LC1 = <131 LC50 = 329 LC1 = 138 LC50 = 385	Birge et al. (1979)
<i>Oncorhynchus mykiss</i>	Embryo-larval-test	Flow-through	12.5 – 14.5	50 200	7.9 – 8.1	27 d	LC1 = <16.9 LC50 = 90.5 LC1 = 20.2 LC50 = 114	Birge et al. (1979)
<i>Pimephales promelas</i>	Generation-cycle test	Flow-through	24 ± 2	34.0 – 45.2	7.6 ± 0.4	224 d	NOEC > 54	Arthur et al. (1974)

(all concentrations related to Na₃NTA and based on analytical measurements)

Macek & Sturm (1973) conducted the acute and long-term toxicity of NTA on different fish species. The test was performed in a flow-through system (detention time 5 h) with a total hardness of 35 mg/l CaCO₃. 96-h-LC50 values of 98 mg/l for *Oncorhynchus mykiss* and 127 mg/l for *Pimephales promelas* were determined. After 28 d exposure, for *Lepomis macrochirus* a LC10 of 96 mg/l and a LC55 of 173 mg/l was determined, while for *P. promelas* the LC0 was 96 mg/l and the LC100 173 mg/l. In the long-term test, both species cumulative mortality due to continuous NTA exposure was conspicuously absent. Examination of gills of fish exposed to 96 mg/l NTA for 28 d indicated no changes in histology.

Birge et al. (1979) conducted an embryo-larval-test with the fish species channel catfish (*Ictalurus punctatus*), goldfish (*Carassius auratus*), and rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*). Each test was performed in a flow-through system (detention time 2.5 h) at two water hardness levels (50 and 200 mg/l CaCO₃). The NTA concentration was monitored daily. Exposure was initiated 20 min after fertilization in trout, 1 to 2 h post-spawning for goldfish, and 2 to 12 h after spawning for channel catfish. Average hatching times were 23, 4.5, and 4 days for trout, catfish, and goldfish, respectively. One test parameter was the egg hatchability, including all embryos (normal or aberrant). Another test parameter was the survival of normal organisms, determined at hatching and 4 days posthatching. Normal organisms were defined as those animals that were free of gross teratic defects. At 4 days posthatching, the Na₃NTA LC50 values were 90.5, 240.4, and 329.3 mg/l for trout, goldfish, and catfish stages exposed in soft water, and 114, 243.4, and 384.7 mg/l in hard water. The LC1 values derived by log probit analysis were <16.9 mg/l (trout), 28.5 mg/l (goldfish), and <131 mg/l (catfish) in soft water, and 20.2, 30.1, and 138.4 mg/l in hard water.

An extended study of the acute and chronic toxicity of NTA on the fathead minnow (*Pimephales promelas*) according to the APHA standard procedure was conducted by Arthur et al. (1974). Water from Lake Superior (hardness about 40 mg/l as CaCO₃) was used as the test medium. Analytical measurements during the test period revealed that NTA was biodegraded (substance loss up to 24%), therefore all referred concentrations are based on the measurements. The short-term toxicity test was conducted in a flow-through test system (retention time 5 h) with juvenile fish, the 96-h LC50 was determined to 114 mg/l Na₃NTA. For the chronic (generation-cycle) test, twenty 3-15-day-old fry were placed in each vessel and exposed to 5 different NTA concentrations (2.1 – 53.9 mg/l Na₃NTA). After 30 days exposure, larval growth was not affected even by the highest tested concentration. After an exposure period of 224 days, there were no observable differences in survival, spawning activity, and egg hatchability at the highest tested concentration of 53.9 mg/l Na₃NTA. At this concentration, NTA is mainly complexed with Ca and Mg. The individual exposure time of single development stages is not definitively specified.

Tests on acute toxicity to fish resulted in 96-h LC50 values in the range of 98 – 487 mg/l. In all of these tests effects were observed when the Na₃NTA concentration exceeded the stoichiometric metal levels (mainly Ca and Mg) in the medium. A generally accepted hypothesis is that the toxicological profile of complexing agents is based on disturbances of metal metabolism. It is expected that effects are caused by the uncomplexed agent. This is supported by the increased effect values in hard water. Even in the 28 d test with adult fish (Macek & Sturm, 1973) the LC0 resp. LC10 values of 96 mg/l are approximately equal to the stoichiometric metal levels.

Lower effect values (LC1 in the range of <16.9 – <131 mg/l) were determined by the embryo-larval tests conducted by Birge et al. (1979). The effect values are usually very low compared

to effect values found by other authors. No explanation for these discrepancies could be found. A careful examination of the entire information provided by Birge et al. gave no plausible reason for the inconsistency of the data. However, as it was not possible to reproduce the effect values, it was decided by the EU member states not to use these data for a derivation of a PNECaqua if other valid fish early life stage tests are available. Therefore, the effect values found by Birge et al. are not employed in the further effects assessment.

In a generation-cycle test over 224 days on *Pimephales promelas* (Arthur et al., 1974), there were no observable differences in survival, spawning activity, and egg hatchability at the highest tested concentration of 54 mg/l Na₃NTA (the active test substance was Ca- resp. Mg-NTA). Based in this study, the NOEC for fish is determined to 54 mg/l.

3.2.1.2 Toxicity to Invertebrates

Because of the ready degradability of NTA, in static tests the test solutions are probably not stable over the total test period. Based on the available monitoring data, it is expected that the stability is guaranteed up to 48 h. Static or semi-static tests over a longer period are possibly not valid and thus not referred here. There is a large database on the toxicity of Na₃NTA on invertebrates, an overview of the results considered to be valid is presented in tables 3.15 and 3.16.

a) Crustacea

An immobilisation test on *Daphnia magna* in a medium with a water hardness of 286 mg/l CaCO₃ was conducted by Bringmann & Kühn (1977a). It is not clear whether H₃NTA or Na₃NTA was used as test substance. The test solution was neutralized (pH 7.6 – 7.7). After 24 h exposure, a EC₀ of 800 mg/l, a EC₅₀ of 950 mg/l, and a EC₁₀₀ of 1350 mg/l were obtained. In a further test (Bringmann & Kühn, 1982) in a similar medium without neutralization, the effect concentrations were EC₀ 75 mg/l, EC₅₀ 79 mg/l, and EC₁₀₀ 83 mg/l. After neutralization (pH 8.0), the EC₅₀ was above 1000 mg/l. The test substance was not monitored, thus all concentrations are nominal. The results indicate that the effects were largely due to the change of the pH value.

A static short-term toxicity test with the crustacea *Daphnia magna* was carried out in a medium with a hardness of 220 mg/l CaCO₃ (Canton & Sloof, 1982). The EC₅₀ value was in the range of 560 – 1000 mg/l for *Daphnia* (endpoint: immobilisation, mortality).

Flannagan (1971) tested the toxicity of Na₃NTA on 17 species of macro-invertebrates using 4 different natural waters with different hardness. Monitoring of the test substance revealed no significant decrease over a period of 73 h. *Hyallela azteci* was tested in a flow-through system in unbuffered water (pH 9.3, 21 mg/l CaCO₃), the 72-h LC₅₀ was above 250 mg/l. Experiments with *Gammarus lacustris* in a static system showed a LC₅₀ of about 600 mg/l in unbuffered hard water. Tests with *Pontoporeia affinis* in a flow-through system, the LC₅₀ was above 1000 mg/l in buffered soft water (21 mg/l CaCO₃)

Table 3.15: Toxicity of Na₃NTA to invertebrates in short-term tests

Species	Method	Test type	Test conditions			Exposure time	Effect [mg/l]	conc.	Nominal / measured	Reference
			Temp [°C]	Hardness [mg/l CaCO ₃]	pH					
Crustacea										
<i>Daphnia magna</i>	Immobilisation test	Static	20 – 22	286	7.6 – 7.7	24 h	EC50 = 950 *	N		Bringmann & Kühn (1977a)
<i>Daphnia magna</i>	Immobilisation test	Static	20	286	ND	24 h	EC50 = 79 *	N		Bringmann & Kühn (1982)
<i>Daphnia magna</i>	Immobilisation test	Static	19 ± 1	220	ND	48 h	EC50 = 560 - 1000	N		Canton & Sloof (1982)
<i>Hyallela azteca</i>	APHA method	Flow-through	10	21	ND	72 h	LC50 > 250	M		Flannagan (1971)
<i>Pontoporeia affinis</i>	APHA method	Flow-through	10	21	Ca. 7.0	96 h	LC50 > 1000	M		Flannagan (1971)
<i>Gammarus lacustris</i>	APHA method	Static	10	74	7.0	96 h	LC50 = ca. 600	M		Flannagan (1971)
<i>Gammarus pseudolimnaeus</i>	APHA method	Flow-through	17 ± 1	ND	7.9 – 9.2	96 h	LC50 = 98	M		Arthur et al. (1974)

*: unclear whether concentrations refer to H₃NTA or Na₃NTA

Table 3.15 contd.

Species	Method	Test type	Test conditions			Exposure time	Effect [mg/l]	conc.	Nominal / measured	Reference
			Temp [°C]	Hardness [mg/l CaCO ₃]	pH					
Molluscs										
<i>Physa heterostropha</i>	APHA method	Static	20 ± 1	60 170	ND	96 h	LC50 = 373 LC50 = 522	M	Weaver (1970)	
<i>Helisoma trivolis</i>	APHA method	Static	Room t.	74	ND	96 h	LC50 > 250	M	Flannagan (1971)	
<i>Physa spec.</i>	APHA method	Static	Room t.	21 745	ND	96 h	LC50 = ca. 400 LC50 = ca. 700	M	Flannagan (1971)	
Insects										
<i>Aedes aegypti</i>	Dutch standard method / mortality	Static	23 ± 2	220	ND	48 h	LC50 => 5600	N	Canton & Sloof (1982)	

Table 3.16: Toxicity of Na₃NTA to invertebrates in long-term tests

Species	Method	Test type	Test conditions			Exposure time	Effect [mg/l]	conc.	Nominal / measured	Reference
			Temp [°C]	Hardness [mg/l CaCO ₃]	pH					
Crustacea										
<i>Gammarus pseudolimnaeus</i>	Generation-cycle test	Flow-through	17 ± 1	35.2 – 45.4	7.8 ± 0.1	147 d	NOEC = 9.3	M	Arthur et al. (1974)	
Snails										
<i>Helisoma trivolis</i>	Reproduction / mortality / growth / fecundity	Flow-through	23	ND	7.4 – 7.5	120 d	NOEC = 12.5	M	Flannagan (1974)	

An extended study of the acute and chronic toxicity of NTA on the amphipod *Gammarus pseudolimnaeus* according to the APHA standard procedure was conducted by Arthur et al. (1974). Water from Lake Superior (hardness about 40 mg/l as CaCO₃) was used as the test medium. Analytical measurements during the test period revealed that NTA was biodegraded (substance loss up to about 50%), therefore all referred concentrations are based on the measurements. Both tests were conducted in a flow-through test system with a retention time of 5 - 6 h. The 96-h LC50 was determined to 98 mg/l Na₃NTA. For the chronic test, 25 individuals of 18-day-old newly hatched young were placed in each vessel and exposed to 5 different NTA concentrations (1.2 – 51.9 mg/l Na₃NTA). Over 21 weeks exposure, the recorded parameters were survival, final gravid females, number of produced young, total births, number of young per female, and births per female. No young or births were produced in the 18.7 and 51.9 mg/l test chambers. The reproduction index (determined by adding total births plus final gravid females divided by final number of surviving females) showed a significant decrease in female fecundity in concentrations \geq 18.7 mg/l. The lowest tested concentration without significant effects was 9.3 mg/l Na₃NTA. At this concentration, NTA is mainly complexed with Ca and Mg.

b) Molluscs

Weaver (1970) conducted short-term tests with the snail *Physa heterostropha* in both soft and hard water. 10 adult snails with an average diameter of 1.0 cm were exposed for 96 h. Monitoring showed essentially no loss of NTA throughout the test, which was not unexpected as the media were either sterile or were made from distilled water. The LC50 values were 522 mg/l in hard water (170 mg/l CaCO₃) and 373 mg/l in soft water (60 mg/l CaCO₃).

Flannagan (1971) tested the toxicity of Na₃NTA on 17 species of macro-invertebrates using 4 different natural waters with different hardness. Monitoring of the test substance revealed no significant decrease over a period of 73 h. *Helisoma trivolis* was tested in both buffered and unbuffered media, in both experiments the LC50 was above 250 mg/l. At higher Na₃NTA concentrations, the toxicity was higher in the unbuffered medium, the lethal effects are probably due to the increase of pH. Experiments with *Physa sp.* resulted in LC50 values of about 400 mg/l in a water hardness of 21 mg/l CaCO₃ and about 700 mg/l in a water hardness of 745 mg/l CaCO₃.

A test on the influence of NTA on mortality, growth and fecundity through 4 generations of the freshwater snail *Helisoma trivolis* was conducted by Flannagan (1974). 10 juvenile snails were exposed to 5 Na₃NTA concentrations in a flow-through system, their weight was measured daily during the 120 d exposure period. 10 snails of the offspring of each concentration group were selected to form the next generation. Monitoring of NTA revealed that the test substance concentration was stable. No significant growth differences were found between snails exposed to 6.25 and 12.5 mg/l Na₃NTA and their controls. At 25 mg/l only the F1 snails were significantly smaller, while with 50 mg/l the F1 and F2 snails but not the F3 generation was smaller than the control groups. At 100 mg/l all four generations were reduced in weight. Lethal effects were not observed up to 100 mg/l. From this test, a NOEC of 12.5 mg/l can be determined.

c) Insects

A static short-term toxicity test with 3-4 weeks old larvae of the insect *Aedes aegypti* was carried out in a medium with a hardness of 220 mg/l CaCO₃ (Canton & Sloof, 1982). The LC50 was in the range of 5600 – 10000 mg/l.

All tests on acute toxicity to invertebrates showed effects only when the Na₃NTA concentration exceeded the stoichiometric metal levels of the medium. It is expected that effects are caused by the uncomplexed agent. This is supported by the increased effect values in hard water.

In long-term tests, the most sensitive organism was the amphipod *Gammarus pseudo-limnaeus*. In a generation-cycle test over 21 weeks exposure, the lowest tested concentration without significant effects was 9.3 mg/l Na₃NTA. Based in this study, the NOEC for invertebrates is determined to 9.3 mg/l. At this concentration, NTA is mainly complexed with Ca and Mg.

3.2.1.3 Toxicity to algae

The influence of medium composition on the growth inhibition of 3 algal species (*Selenastrum capricornutum*, *Scenedesmus subspicatus*, *Chlorella vulgaris*) was examined by Millington et al. (1988). Bold's Basal medium (BBM) is a very rich medium containing much higher concentrations of nutrients compared to OECD and EPA media. The method used followed the OECD test guideline. NTA (unclear whether acid or sodium salt) was tested at 5, 10, 50, 80, and 100 mg/l. The 5d-NOECs (related to cell concentration) are 5 mg/l for all 3 species in both OECD and EPA medium, while 50 mg/l (*S. capricornutum*) and 80 mg/l (*S. subspicatus*, *C. vulgaris*) for BBM was obtained. The test results indicate that the apparent effects are mainly caused by nutrient deficiency.

Both static and continuous flow tests on growth inhibition of the diatom *Navicula seminulum* using hard and soft nutrient solution was conducted by Weaver (1970). Test cultures were prepared by placing diatom stock solution onto millipore filters and introducing the filters into flasks containing nutrient solutions. At the conclusion of each test the cultures were dried and weighed. Monitoring throughout the tests showed essentially no loss of NTA. In the static test, the 96h-EC_{b50} were 477 mg/l for hard water and 185 mg/l for soft water. Similar results were obtained in the flow-through system, the 96h- EC_{b50} were 477 mg/l for hard water and 133 mg/l for soft water. In both media, the concentrations of nutrient metals (e.g. 2 mg/l ZnSO₄ or 1 mg/l CoCl₂) were relatively high thus preventing nutrient deficiency.

A static growth inhibition test on *Chlorella vulgaris* and *Microcystis aeruginosa* was conducted by Canton & Slooff (1982). The 96h-EC_{b50} for *C. vulgaris* is in the range of 560-1000 mg/l and for *M. aeruginosa* in the range of 180 – 320 mg/l Na₃NTA. The concentrations of nutrient metals (e.g. 110 µg/l ZnCl₂ or 80 µg/l CuSO₄) in the test medium were relatively high thus preventing nutrient deficiency.

Table 3.17: Toxicity of Na₃NTA to algae in growth inhibition tests

Species	Medium / Test type	Test conditions			Exposure time	Effect conc. [mg/l]	Nominal / measured	Reference
		Temp [°C]	Hardness [mg/l CaCO ₃]	pH				
<i>Selenastrum capricornutum</i>	BBM /static	22°C	ND	ND	5 d	NOEC = 50 mg/l	N	Millington et al. (1988)
	OECD /static					NOEC = 5 mg/l		
	EPA /static					NOEC = 5 mg/l		
<i>Scenedesmus subspicatus</i>	BBM /static	22°C	ND	ND	5 d	NOEC = 80 mg/l	N	Millington et al. (1988)
	OECD /static					NOEC = 5 mg/l		
	EPA /static					NOEC = 5 mg/l		
<i>Chlorella vulgaris</i>	BBM /static	22°C	ND	ND	5 d	NOEC = 80 mg/l	N	Millington et al. (1988)
	OECD /static					NOEC = 5 mg/l		
	EPA /static					NOEC = 5 mg/l		
<i>Nacicula seminulum</i>	APHA /static	20 ± 1	60 170	ND	96 h	EC50 = 185 mg/l EC50 = 477 mg/l	M	Weaver (1970)
<i>Nacicula seminulum</i>	APHA / flow through	20 ± 1	60 170	ND	96 h	EC50 = 133 mg/l EC50 = 477 mg/l	M	Weaver (1970)
<i>Chlorella vulgaris</i>	static	22 ± 2	220	ND	96 h	EC50 > 560 mg/l	N	Canton & Sloof (1982)
<i>Microcystos aeruginosa</i>	static	23 ± 2	220	ND	96 h	EC50 > 180 mg/l	N	Canton & Sloof (1982)
<i>Cyclotella nana</i>	static	20 ± 1	ND	8.2	72 h	LOEC = 1 mg/l	N	Erickson et al. (1970)

The effect of NTA on the marine algae *Cyclotella nana* using synthetic seawater was studied by Erickson et al. (1970). Growth rates (determined as cell density) were determined with 0.25, 0.5, 1.0, 2.5, and 5.0 mg/l Na₃NTA after 72h. The nutrient metal concentrations are comparable with the OECD standard medium. Addition of 0.25 to 0.5 mg/l Na₃NTA resulted in greater growth at 8.5°C, but not at 2.0°C. The addition of 1.0 to 5.0 mg/l resulted in a progressive inhibitory effect with time (EC₅₀ about 2.5 mg/l) at both temperatures. The inhibitory effect is attributed to the reduced bioavailability of trace metals.

The apparent toxicity of complexing agents to algae in standard tests is related to essential trace metal bioavailability. Trace metal levels tend to be more important in algal growth tests than in other short-term tests (e.g. on fish or daphnia); the main reason is the rapid increase of biomass during the test. In standard tests using uncomplexed agents, the concentrations of free essential metal ions decrease drastically, leading to nutrient deficiency and relatively low effect concentrations. Addition of higher amounts of nutrient metals result in detoxification of the agent. This effect is also known from other complexing agents, e.g. EDTA (Dufková, 1984) and [S,S]-Ethylenediamine disuccinate, [S,S]-EDDS (Schowanek et al., 1996), with both substances the apparent toxicity disappeared when stoichiometric amounts of the nutrient metals were added.

3.2.1.4 Effects on ecosystems – pond studies

The influence of 100 and 500 µg/l NTA (as H₃NTA) on the biocoenosis of pond ecosystems was investigated by Hamm (1991), and Kucklantz (1991). Eight experimental ponds were installed at a test field in Wielenbach, Germany. Each pond had a surface area of 1700 – 2000 m² and contained approximately 1400 m³ water. The ponds simulated a natural system with macrophytes and littoral vegetation, stocked with fresh water crayfish, 50 carps, 20 grassfish and 20 tenches (*Tinca tinca*). The winter population in all ponds was smaller than in summer. Two types of ponds were used, one having a minimal water exchange and a retention time of ca. six weeks, whereas the other type was equipped with a water-batcher, providing a continuous inflow of the test substance, with a retention time of exact two weeks. An average concentration of 100 µg/l NTA was measured in the stagnant pond while the two NTA flow ponds exhibited average inflow concentrations of 100 and 500 µg/l NTA, respectively. Phosphate (10 & 100 µg/l) and copper (50 µg/l), known as accelerating and inhibiting primary production served as positive controls. One pond of each type was not charged with test substances and served as negative control. One control and one test pond of each type is used. The experiments took place over two years (i.e., two vegetation periods). It was concluded that there was no significant difference in macrophytes, concentration of chlorophyll a, zoobenthos, zoo- or phytoplankton between the control ponds and the ponds with 100 or 500 g/l H₃NTA (i.e. 135 and 670 mg/l Na₃NTA) in summer or winter. No toxic effects were noted in the fish at both NTA levels. No remobilization of metals took place.

3.2.1.5 Influence on the toxicity of heavy metals

The uptake of heavy metals by aquatic organisms is strongly dependent on the chemical speciation of the metal in the environment. Van Ginneken et al. (1999) demonstrated that NTA decreased the uptake of the radioisotopes ^{109}Cd and ^{65}Zn by the carp *Cyprinus carpio*. The presence of NTA (0.01 – 0.1 $\mu\text{mol/l}$) decreases the concentrations of free metal ion activity, thus the uptake was reduced.

Erickson et al. (1970) studied the influence of NTA on the toxicity of copper to the marine algae *Cyclotella nana*. In a growth inhibition test, extreme inhibition occurred after addition of 50 $\mu\text{g/l}$ Cu, in two replicates growth was reduced to 11 resp. 17.5% of the control after 72 h. Further addition of 0.25 to 5.0 mg/l H_3NTA reduced the growth inhibition, and a concentration of 0.5 mg/l was sufficient to nullify copper toxicity.

3.2.1.6 Derivation of PNECaqua

According to the results from different ecotoxicological studies discussed above, the toxicological profile of NTA is based on disturbances of metal metabolism. For the interpretation of toxicity tests, the complex formation properties of NTA have to be taken into account. In section 3.1.2.3, the main features on complex chemistry in the environment are elaborated. The reactions in the test media are comparable.

Beside Ca and Mg, test media contain a certain amount of heavy metal ions being necessary as trace nutrients. The complex forming constants of heavy metal complexes are by several orders of magnitude higher than of Ca/Mg-complexes, thus after addition of the test substance NTA (as acid or Na-salt) the concentration of uncomplexed trace metals decreases drastically. The degree of Ca/Mg complexation is dependent on the amount of added NTA. Uncomplexed NTA is only present when it is present in over-stoichiometric concentrations.

The choice of the complex species being relevant for effect testing should consider the environmental relevance. Effect tests should be conducted with a complex for which metal toxicity can be excluded. As shown in section 3.1.2.3, always a mixture of metal complexes is released resp. being formed in surface waters. Using the Ca-complex as test substance appears to be appropriate, as it is probably the predominant species in freshwater systems.

In tests on acute toxicity to fish effects were observed when the Na_3NTA concentration exceeded the stoichiometric metal levels (mainly Ca and Mg) in the medium. It is expected that effects are caused by the uncomplexed agent. In surface waters, always over-stoichiometric amounts of metal ions are present, thus the available tests are not relevant for environmental conditions. Even in the 28 d test with adult fish (Macek & Sturm, 1973) the LC0 resp. LC10 values of 96 mg/l are approximately equal to the stoichiometric metal levels.

In a generation-cycle test on *P. promelas* over 224 days (Arthur et al., 1974), there were no observable differences in survival, spawning activity, and egg hatchability at the highest tested concentration of 54 mg/l Na₃NTA. At this concentration, NTA is mainly complexed with Ca and Mg. Based in this study, the NOEC for fish is determined to 54 mg/l.

Similar to the fish tests, all tests on acute toxicity to invertebrates showed effects only when the Na₃NTA concentration exceeded the stoichiometric metal levels of the medium. In hard water, the effect values are increased. It is expected that effects are caused by the uncomplexed agent, thus the available tests are not relevant for environmental conditions. In long-term tests, the most sensitive organism was the amphipod *Gammarus pseudolimnaeus*. In a generation-cycle test over 21 weeks exposure, the lowest tested concentration without significant effects was 9.3 mg/l Na₃NTA. At this concentration, NTA is mainly complexed with Ca and Mg. Based in this study, the NOEC for invertebrates is determined to 9.3 mg/l.

The apparent effects of complexing agents to algal growth is related to essential trace metal bioavailability. Trace metal levels tend to be more important in algae tests than in short-term tests on fish or daphnia, the main reason is the rapid increase of biomass during the test. The effect concentrations increased with the trace metal amounts. The test results indicate that not the absolute NTA concentration, but rather the ratio of the NTA to the metal cation concentration is crucial to algae growth. In media with low trace metal concentrations like the OECD standard medium, effects were observed in the range of 1 – 5 mg/l, while in metal-enriched media the NOECs were ≥ 50 mg/l.

The apparent toxicity of complexing agents to algae can be caused either by its intrinsic toxicity or by indirect effects like nutrient deficiency. The studies cited above reveal that the effects are mainly caused by the latter. Therefore, these inhibition of algae growth is an artefact which is caused by the drastic increase of biomass during the test. Such indirect effects cannot be quantified from the laboratory tests, thus only theoretical considerations can be made. In German and Dutch rivers, heavy metal concentrations in the range of 10-20 $\mu\text{mol/l}$ (predominantly Fe and Mn) are detected. The stoichiometric Na₃NTA equivalent, i.e. the NTA amount needed for complete complexation of the heavy metals, is 2.6 – 5.1 mg/l. Estimations of the speciation in the hydrosphere show that the largest NTA fraction is complexed with Ca, therefore complete complexation of heavy metals is expected only with extremely high NTA concentrations. As in the environment metal ions are generally present in over-stoichiometric amounts, nutrient deficiency is not expected. Nutrient deficiency in surface waters could only occur when essential metal ions are over-chelated. Furthermore, plant growth is influenced by many limiting parameters, probably the presence of macronutrients like phosphate or nitrate is of greater importance.

In addition to the discussed adverse effects like growth inhibition and nutrient deficiency, growth stimulating effects like eutrophication may occur. The presence of a chelator can improve the bioavailability of nutrient metals. Also this effect can only qualitatively be assessed. In the environment, higher availability of trace elements through the complexing agent depends on the preloading of the water and could stimulate the

processes of eutrophication. If trace elements like Fe, Co, Mn, and Zn are sufficiently available in a soluble form, the plants growth will not be influenced. Because of the presence of over-stoichiometric amounts of heavy metals, it is unlikely that eutrophication is caused by NTA.

Besides the monospecies tests used for the PNEC determination, a study on pond ecosystems (Kucklantz, 1991; Hamm, 1991) is available. With concentrations up to 500 µg/l H₃NTA (= 670 µg/l Na₃NTA) neither nutrient deficiency nor eutrophication was observed.

The effects assessment of NTA is based on long-term tests, which are available for fish, daphnids and algae. The most sensitive endpoint was found for the amphipod *Gammarus pseudolimnaeus* with a NOEC of 9.3 mg/l. According to TGD an assessment factor of 10 has to be used. Therefore, a **PNECaqua of 0.93 mg/l** is determined.

3.2.1.7 Toxicity to Microorganisms

A series of monospecies tests to microorganisms is available, an overview is presented in table 3.18.

Table 3.18: Toxicity of Na₃NTA to microorganisms

Species	Method	Test type	Test conditions			Exposure time	Effect [mg/l]	conc.	Nominal / measured	Reference
			Temp [°C]	Hardness [mg/l CaCO ₃]	pH					
Bacteria / Cyanobacteria										
<i>Pseudomonas fluorescens</i>	Cell multiplication inhibition / biomass	static	23 ± 2	220	ND	8 h	EC50 = 3200-5600	N		Canton & Sloof (1982)
<i>Pseudomonas putida</i>	Cell multiplication inhibition / biomass	static	27	ND	7.0	16 h	EC5 > 10000	N		Bringmann & Kühn (1977b)

Protozoa									
<i>Chilomonas paramecium</i>	Cell multiplication inhibition / biomass	static	20	ND	6.9	72 h	EC5 > 540	N	Bringmann et al. (1980)
<i>Entosiphon sulcatum</i>	Cell multiplication inhibition / biomass	static	20	ND	6.9	72 h	EC5 > 1100	N	Bringmann (1978)
<i>Uronema parduzci</i>	Cell multiplication inhibition / biomass	static	20	ND	6.9	72 h	EC5 > 1100	N	Bringmann & Kühn (1980)

a) Bacteria

A static growth inhibition test on *Pseudomonas fluorescens* was conducted by Canton & Slooff (1982). The tested bacteria were in the log phase at the start of exposure. The medium contained 5,000 mg/l glucose as substrate. The 96h-EC50 values are in the range of 3200 - 5600 mg/l.

Bringmann & Kühn (1977b) tested the inhibition of cell multiplication with *Pseudomonas putida*. No effects were observed in concentrations up to 10,000 mg/l H₃NTA (= 13,500 mg/l Na₃NTA) after 16 h of exposure.

b) Protozoa

A test on cell multiplication inhibition with different protozoa was performed using identical experimental conditions. Stock and preliminary cultures of the test organisms were fed with living bacteria, whereas the test cultures were fed with inactivated bacteria. H₃NTA was used as test substance. After 72 h exposure, the toxic threshold concentrations (EC5) were > 400 mg/l H₃NTA (> 540 mg/l Na₃NTA) for *Chilomonas paramecium* (Bringmann et al., 1980), > 800 mg/l H₃NTA (> 1100 mg/l Na₃NTA) for *Entosiphon sulcatum* (Bringmann, 1978), and > 800 mg/l H₃NTA (> 1100 mg/l Na₃NTA) for *Uronema parduzci* (Bringmann & Kühn, 1980).

Determination of PNEC_{micro-organism}

There are tests on different microorganisms available. Similar to the algae tests, it cannot be excluded that the apparent effects in these tests were caused by nutrient deficiency by reduction of the concentrations of essential metals. It is expected that the intrinsic toxicity of NTA is lower. The available test results can be used as a worst case approach.

The lowest effect value obtained in a test with bacteria is > 3200 mg/l for *Pseudomonas fluorescens*. For the PNEC derivation this value is not employed as glucose was used as substrate. The lowest effect value from protozoa tests is a 72h-EC5 of > 540 mg/l (as Na₃NTA) for *Chilomonas paramecium*. In accordance to the Technical Guidance Documents, an assessment factor of 1 is applied.

→ PNEC_{microorg.} > 540 mg/l

3.2.2 Atmosphere

Because there are no fumigation tests available, an effects assessment for this compartment can not be performed.

3.2.3 Terrestrial compartment

There are no tests on terrestrial organisms available, thus an effects assessment for this compartment can not be performed.

3.2.4 Non compartment specific effects relevant to the food chain

As there is no bioaccumulation, a biomagnification via the food chain is not expected.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

The risk assessment for aquatic organisms resulted in a PNECaqua of 0.93 mg/l. The PNECmicroorg. was determined to >540 mg/l.

In the following table, the results for all calculated exposure scenarios are listed:

Table 3.19: Risk characterization for the aquatic compartment

Scenario	PEC _{local} _{aqua} [µg/l]	PEC _{aqua} / PNECaqua	Ceffl [mg/l]	Ceffl. / PNEC _{micro}
Producer A	only import			
Producer B	< 5.1	< 0.005	No wwtp	
Producer C	5.6	0.006	0.054	< 0.0001
Producer D	< 450	< 0.48	No wwtp	
Producer E	14	0.015	1.1	< 0.002
Textile Cleaning, Formulation	20	0.022	0.16	< 0.0003
Textile Cleaning, Use	500	0.54	5.0	< 0.009
Cleaning agents, Formulation	49	0.053	0.45	< 0.0008
Cleaning agents, Use	49	0.053	0.45	< 0.0008

The available studies on biodegradation reveal that NTA is removed in municipal treatment plants with rates generally above 95% under normal operation conditions. Contradicting results were obtained for measurements in the winter season: while in several cases no difference between summer and winter was observed, other studies show considerable decrease (<50 %) at low temperatures. For the exposure calculations in this assessment, a removal of 95% was used. Assuming in a worst case approach a removal rate of 50%, only the PEC/PNEC ratio of the scenario for the use in textile cleaning will be > 1 (2,0). Taking into account, that this scenario is nearly completely based on default values, it is concluded that even with a lower biodegradation there is no risk to the aquatic compartment.

Sediments

There are neither monitoring data for sediments nor toxicity tests with benthic organisms available. The Na_3NTA concentration could be modelled using the equilibrium partitioning method. A risk assessment for sediments would lead to identical PEC/PNEC ratios like for the aquatic compartment.

Because of the low partitioning coefficients, no accumulation in sediments is expected. Thus an assessment of this sub-compartment is not necessary.

Influence on the Distribution of Heavy Metals

In section 3.1.2.3 the influence of NTA on the distribution of heavy metals was examined. It was concluded that significant remobilization processes are only expected in extreme cases, i.e. when high NTA amounts are released. This would lead to increased concentrations of those metals with high conditional complex-formation constants. With the concentrations estimated in this risk assessment, those effects are not expected.

- ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

3.3.2 Atmosphere

No relevant releases into the atmosphere are expected; therefore a risk characterisation for this compartment is not necessary.

3.3.3 Terrestrial compartment

No relevant releases into soils are expected; therefore a risk characterisation for the terrestrial compartment is not necessary.

3.3.4 Non compartment specific effects relevant to the food chain

As there is no bioaccumulation, a biomagnification via the food chain is not expected.

- ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Trisodium nitrilotriacetate (Na_3NTA) is produced and /or imported to the European Union (EU) by five companies. According to CEFIC (2001) approx. 27.000 t/a were marketed in 2000. The chapter 2 goes into details according the producer data.

Nitrilotriacetic acid and trisodium nitrilotriacetate are often used to soften water and to remove traces of alkaline earth and heavy metals.

From the total European consumption of trisodium nitrilotriacetate around 67 % are applied in cleaning agents for household and industrial use, 29 % of the marketed amount are used in other industrial categories e.g. in miscellaneous uses as water softener, about 3.7 % are used for the textile cleaning in industry and household (CEFIC, 2001).

Industrial applications of formulations are listed below, most are mentioned in the IUCLID database (IUCLID, 1996):

- water treatment (chelating agent)
- rubber processing (Oekopro 6.0, 2003)
- textile industry
- paper industry
- chemical laboratories
- photographic materials.

The results of the search for trisodium nitrilotriacetate in the Danish Product Register of December 2006 list 486 products containing trisodium nitrilotriacetate with a total tonnage of 399 t/a (Danish Product Register 2006). The product types are cleaning/washing agents, complexing agents, preservatives, stabilisators, ph-regulating agents and surfactants.

In the Swedish product register and in the BfR data base for consumer products, Na_3NTA is listed as an ingredient in a number of products, namely in detergents and cleaners.

According to information from BASF (Technical Bulletin) Na_3NTA is used for the following applications:

- in detergents to ensure that surfactants remain free to participate in the washing process

- in cleaners/degreasers to prevent water-based formulations from becoming cloudy or precipitating; to prolong the working life of degreasing bath
- in textile finishing by softening water and sequestering heavy metal ions to prevent solids from precipitating when fabrics are boiled off.

These applications can lead to the dermal, inhalation and oral exposure of the consumer. The routes of exposure are by inhalation of dust and by skin contact at the workplace.

4.1.1.2 Occupational exposure

The exposure assessment generally aims at assessing exposure levels representing the reasonable worst case situation. The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for a specific scenario.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 95th percentiles are derived as representing reasonable worst case situations. For the purpose of exposure assessment up to date data are not available. If quantitative exposure data are not available, model estimates are used. Therefore scenarios are clustered as far as possible to make the description transparent.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:

Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.

Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.

Within the framework of existing substances there is an agreement between the EU member states, to assess – as a rule – dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure-reducing effect achievable by gloves is only considered if information is provided indicating that, for a certain scenario, gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as a criterion. For most downstream uses it is commonly known that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since quantitative information on dermal exposure is often not available, the EASE model is mostly used for assessing dermal exposure.

No occupational limits are given.

The following scenarios are regarded to be relevant for occupational exposure:

Production of trisodium nitrilotriacetate (Na₃NTA)

Use of trisodium nitrilotriacetate in formulation processes

Uses of formulations

Trisodium nitrilotriacetate is a white crystalline powder and is mainly handled in the form of aqueous preparations. Due to the physico-chemical properties of the substance (solid at room temperature, low vapour pressure (< 1 Pa)), inhalation exposures to vapour during the handling of solutions without formation of aerosols is assumed to be negligible. With regard to the handling of powdery trisodium nitrilotriacetate, exposures to dust in the production of the substance and its further processing to aqueous solutions during charging activities and filling activities are mainly to be expected. There is no information about the particle diameter. Exposures to droplet aerosols during high-pressure application of aqueous preparations may additionally occur.

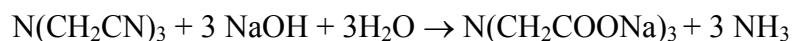
4.1.1.2.1 Scenario 1: Production of trisodium nitrilotriacetate (Na₃NTA)

Today the original synthesis of trisodium nitrilotriacetate from ammonia and chloroacetic acid has only historical significance. The oxidation of triethanolamine is likewise of no industrial importance. The alkaline process was a long time the established method for trisodium nitrilotriacetate production.

The one-stage alkaline and two-stage acid processes now in use are based on the cyanomethylate ion of ammonia (or ammonium sulphate) with formaldehyde and sodium cyanide (or hydrogen cyanide). The production of both forms of trisodium nitrilotriacetate is realized in a closed system.

Alkaline process:

In the first step triscyanomethyl amine is synthesized in a closed system (80°C, without pressure) from ammonia and sodium cyanide with formaldehyde and the presence of sulphuric acid as catalyst. The solid triscyanomethyl amine is then filtered off, washed and saponified with sodium hydroxide to give trisodium nitrilotriacetate.



The reaction can be carried out batch wise or continuously, but the continuous process is more economical.

The resulting solution is sold directly as a 40-wt% solution in drums, containers or rail tanks, or used in the production of trisodium nitrilotriacetate (Na₃NTA) in powder form, or acidified to pH 1 – 2 to yield the acid (H₃NTA).

Acid Process:

The significant yield of by-products in the alkaline process has led in recent years to the construction of plants on the acid process, which features much lower by-products levels. The acid process is associated with stringent safety requirements due to the use of hydrogen cyanide. In the first stage, ammonia is reacted with formaldehyde to give hexamethylenetetramine, which is then reacted with hydrogen cyanide in sulphuric acid solution to yield triscyanomethyl amine. The solid triscyanomethyl amine is sparingly soluble in the acidic solution and is filtered off, washed, and saponified with sodium hydroxide to give trisodium nitrilotriacetate. The resulting solution has a far lower by-product content than the solution from the alkaline method. It is sold as 40 % product or used in the production of trisodium nitrilotriacetate (Na_3NTA) or H_3NTA (see above).

Trisodium nitrilotriacetate is produced in two forms:

- an aqueous solution with a maximum concentration of 40 % and
- as powder

Exposure to trisodium nitrilotriacetate (Na_3NTA) is possible by sampling, cleaning / maintenance, and during filling of the substance in powder form in sacks and big-bags and also by handling the liquid product.

Exposure associated with transporting the chemical would result from loading, unloading, coupling, uncoupling and drumming operations.

For the large-scale chemical industry high standards of control at the workplace are assumed to be practiced even if the containment is breached, e.g. during filling, cleaning, maintenance, repair works and taking of samples. Inhalation exposure in other fields is normally minimized by technical equipment (e.g. special designed filling stations, local exhaust ventilation LEV).

Inhalation Exposure

Workplace measurements

The following table shows the occupational exposure figures in different working areas of the production of trisodium nitrilotriacetate. Data were provided by two companies.

Table 4.1.1.2.1: Trisodium nitrilotriacetate exposure at workplaces in the chemical industry (production: the alkaline process)

Job category / activities	Years of measurement	Number of samples	Range of measurement data [mg/m ³]	Mean [mg/m ³]	90 th -percentile [mg/m ³]	95 th -percentile [mg/m ³]
8h time-weighted average						
Sack-filling	1979-1997	24	0.02 – 5.6	1.3	-	3.9
mixing	1979-1997	4	0.24 – 3.7	1.6	-	-
				Median		
Spray drying (Protok. Rhone Poulenc)	-	16	0 – 0.67	0.16	-	-

For the purpose of measuring trisodium nitrilotriacetate in the air at the workplace the gravimetric sampling method for the inhalable dust fraction was used and the measurements were realized with the BIA-inhalable dust sampler. The measurements of total dust were performed with personal and stationary samples.

There is a measurement collective from 1979 – 1997 for different workplaces. The companies have only exposure data from the sack-filling and mixing area although exposure is possible during sampling, maintenance and repair works. Exposure data from these areas are not available and also no data from the container/tank filling area.

The working places are equipped with local exhaust ventilation. Because of only a few measured values from only two companies a model estimation is performed.

EASE estimation

EASE for Windows 2.0, Aug.1997 was used.

EASE estimation for the production of trisodium nitrilotriacetate in powder form in the large-scale chemical industry:

Input parameters: T = 20°C, dry manipulation, LEV (local exhaust ventilation) present, non-aggregating dust, vapour pressure not determined

Level of exposure 2 – 5 mg/m³

Conclusions

Exposure is possible during filling of the substance in powder form in sacks and big-bags and by sampling, cleaning/maintenance.

The inhalation exposure at handling of liquid trisodium nitrilotriacetate (solution 40%) is not considered because of a low vapour pressure of the substance and the formation of aerosols is regarded to be non-probable.

The workplace measurement value for the powder form - the 95th percentile - and the result of the EASE estimation show the same order of magnitude.

An exposure to trisodium nitrilotriacetate (Na₃NTA) is possible by sampling, cleaning / maintenance and during filling of the substance in powder form in sacks and big-bags.

For the assessment of risks of inhalation exposure to trisodium nitrilotriacetate 3.9 mg/m³ (95th percentile) should be taken for all areas.

Dermal exposure

For the dermal exposure it is relevant that trisodium nitrilotriacetate is produced in two forms: an aqueous solution with a concentration of 40% and as powder.

Dermal exposure is possible during the drumming of the 40 % aqueous solutions and during bagging of trisodium nitrilotriacetate in powder form.

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in a closed system and that the use of PPE (personal protective equipment) (here gloves and eye protection) is highly accepted in the large-scale chemical industry. The extent of protection by PPE (personal protective equipment) (here gloves) depends inter alia on the suitability of recommended material with regard to the permeation properties of substance. For the handling of powdery and aqueous solution, as a rule, the suitability of the gloves can be assumed and the low levels of daily dermal exposure are to be expected. However, in spite of this, dermal exposure may occur due to e.g.

- unintended contamination during the handling of used gloves
- Limited protection of suitable gloves at real working conditions (e.g. mechanical stress).

Since no measurement results are available, an attempt is made to quantify dermal exposure for the above mentioned situations in application of the EASE model. Taking into account that intermediate dermal contact may occur, however, this situation could be described by the scenario:

Input parameters: Non dispersive use, direct handling, intermittent

Level of exposure: 0.1 – 1 mg/cm²/day.

The EASE estimation is similar for liquid or solid trisodium nitrilotriacetate, the difference is the exposed area only.

Referring to the substance in powder form (bagging of solids):

Considering an exposed area of 420 cm² (palms of hands) the model yields an exposure level of 42 – 420 mg/person/day. Taking into account a protection efficiency of the used gloves of 90% dermal exposure is reduced to 4.2– 42 mg/person/day.

Referring to the substance as solution (drumming of liquids):

Considering a concentration of 40 % of the trisodium nitrilotriacetate solution and the possible exposed surface of the hands (210 cm², equivalent to half of one hand) and the 90 % efficiency of the gloves, a predicted dermal exposure of 0.84 – 8.4 mg/person/day is calculated.

Conclusions

For assessing the health risks from daily dermal exposure in the production area handling of trisodium nitrilotriacetate powder and the 40% solution of trisodium nitrilotriacetate is considered.

For trisodium nitrilotriacetate powder the dermal exposure level is 4.2 – 42 mg/person/day. For assessing the risk the upper value of 42 mg/person/day is regarded to represent the reasonable worst case.

The 40 % solution is filled in drums or tanks. Taking into account that the concentration is 40 % dermal exposure is estimated to 8.4 mg/person/day.

These exposure levels are based on the information that suitable gloves are worn and that the dermal contact will be minimised.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Scenario 2: Use of trisodium nitrilotriacetate (Na₃NTA) in formulation processes

In this section, the production of powdery and liquid products containing trisodium nitrilotriacetate (Na₃NTA) is described.

Trisodium nitrilotriacetate is often included in detergent and cleaner formulations for household or industrial use. The application volumes were (CEFIC, 2000; CEFIC 2001):

	Germany (1999)	Western Europe (2000)
Cleaning / washing agents	2,207 t (65 %)	17,905 t (67,2 %)
Textile cleaning, household and industrial	238 t (7 %)	
Others	951 t (28 %)	7,764 t (29,1 %)

These products are applied in different sections:

- water treatment
- rubber processing
- textile industry
- paper industry
- chemical laboratories
- photographic materials.

In the following the production of cleaning/washing agents is exemplary described.

The raw ingredients are stored in bins or tanks and are conveyed through a closed system to reactors and mixers. The mixing process takes approx. 45 minutes. The received slurry is sprayed from the mixer into drying towers. After the drying process the finished powder is conveyed through lines and is automatically filled in bags. The mixing, filling and packing production lines can be a source of dust contamination.

For the liquid cleaning/washing agents the raw materials are pumped from storage tanks into the mixer and after this working step the liquid product is filling into bottles and packed automatically.

In some companies it is possible that trisodium nitrilotriacetate in powder form is added manually (emptying a sack) into a stirred reactor containing an aqueous solution. This is a source for inhalation exposure.

The finished cleaning/washing agents occur in powder form and as liquid. The concentrations of trisodium nitrilotriacetate in the formulations are very different. The area of concentration of trisodium nitrilotriacetate in liquid cleaning/washing agents varied from 0.2 % up to 40 % (BASF, 1997; Monsanto, 1995).

It is to be assumed, that the preparations are produced in specialised formulation companies or at the user site. Possibilities of inhalation and dermal exposure exist during sampling, mixing, charging, filling and packing of the powdery substance. It must be assumed that in small and medium sized companies these activities are performed without local exhaust ventilation and without the use of suitable personal protective equipment (Voullaire, Kliemt, 1995). According to information provided by the industry and the monitoring authorities of the Federal States of Germany, the duration and frequency of the activities of relevance to exposure vary widely. Mostly durations far below 1 hour are given. Sometimes, tasks are performed repeatedly during the given duration.

Taken all the individual information into account, it is derived that the daily duration is one hour at the most.

During the use of trisodium nitrilotriacetate solutions for the preparation of formulations, on account of the physico-chemical properties of the substance, inhalation exposure to vapour is assessed as negligible. Therefore only the inhalation exposure against dust is considered.

Inhalation Exposure

Workplace measurements

One producer has provided workplace measurements from the mixing area of trisodium nitrilotriacetate powder. The solid substance trisodium nitrilotriacetate filled in sacks was manually emptied into a stirred reactor to produce a formulation of cleaning/washing agents. This mixing area is equipped with local exhaust ventilation. It is a measurement collective from 1979 – 1997. The total dust concentration was in the range of 0.24 – 3.7 mg/m³ (n = 4), the mean is 1.6 mg/m³. Further information is not available. Because of only a few measured values from one company the data are not representative and model estimation is performed.

EASE estimation

EASE for Windows 2.0, Aug.1997 was used.

EASE estimation for the use of trisodium nitrilotriacetate in formulation processes:

a) handling the powdery substance with LEV

Input parameters: T = 20°C, dry manipulation, LEV (local exhaust ventilation) present, non-aggregating dust

Level of exposure: 2 – 5 mg/m³

b) handling the powdery substance without LEV

Input parameters: T = 20°C, dry manipulation, LEV (local exhaust ventilation) absent, non-aggregating dust

Level of exposure: 5 –50 mg/m³

Conclusions

There are only a few measurement values from one company in the range that the EASE model estimates. Since no representative measurement results of the formulation of preparations are available, an estimation of the exposure is undertaken using the EASE model.

It is expected that these activities are performed with local exhaust ventilation. The duration of this work adds up to 1 hour per day therefore the level of exposure is 0.62 mg/m³ with LEV.

The level of exposure (b) for the working area without LEV is 6.25 mg/m³. These values are assessed by EASE and in consideration of the working time of 60 minutes.

Dermal exposure

Dermal exposure is possible by filling the mixing reactor to produce the formulation and by handling the formulations containing trisodium nitrilotriacetate. A dermal contact with the solution is possible via connecting a line during the filling of bags or bottles.

For the dermal exposure estimation two situations are considered. First is dumping of powdery trisodium nitrilotriacetate and second is handling of a 40 % aqueous trisodium nitrilotriacetate solution.

For the estimation published reasonable worst case (RWC) and typical default values are available in the literature (Marquart et al., 2006). For loading of mixers with powders at the production of liquids or solid formulations (cleaning, washing agents) a RWC-value of 3000 mg (90th percentile of measurement data) and a typical value of 900 mg are given. The dermal exposure level is estimated to 3000 mg/peron/day (RWC) and 900 mg/person/day /typical).

The second dermal exposure situation is filling and dosing of the 40 % aqueous trisodium nitrilotriacetate solution. Dermal exposure at filling the finalized products is not considered due to the low concentration of trisodium nitrilotriacetate. In the literature (Marquart et al., 2006) described default values for loading of mixers with liquids are 11500 mg (RWC) and 410 mg (typical). Taking into account that the concentration is 40 % the dermal exposure level is estimated to 4600 mg/person/day (RWC) and 164 mg/person/day (typical).

The dermal exposure is assessed for the unprotected worker.

4.1.1.2.3 Scenario 3: Uses of formulations

The formulations containing trisodium nitrilotriacetate are mainly cleaning/washing agents for household and industry.

There are two forms of formulations containing trisodium nitrilotriacetate: liquid and powdery. No specifications about distribution percentage between the forms of formulations are available.

Liquid formulations containing trisodium nitrilotriacetate are used as well in industry as in household sectors. The applications can be subdivided into activities with and without the formation of aerosols.

Use of cleaning products with high pressure equipment

In the area where laundry and cleaning products with high pressure equipment are used the inhalation exposure of aerosols is possible.

The concentrated cleaners containing up to 25 % trisodium nitrilotriacetate are used in high-pressure cleaning equipment. The concentrated cleaners are only used directly in rare exceptional cases. In general diluted solutions are taken for the purpose of washing and cleaning. As a rule, the trisodium nitrilotriacetate concentrations in the cleaning solutions amount to 0.1 % - 2 %.

Use of cleaning agents

Trisodium nitrilotriacetate may be used for all-purpose cleanser by professional cleaners. The concentration in product before dilution amounts to 15 % (see chapter, 4.1.1.3). All-purpose

cleansers are commercially applied to clean surfaces, e.g. floors and windows within buildings. In general aqueous solutions are used. A dilution factor of 100 is assumed (see chapter, 4.1.1.3). In particular cases for very dirty layers the cleansers are used undiluted.

The available information indicates that this product types are not used by spraying. Based on the available information on the processes, aerosols are normally not formed at the workplaces. At workplaces where aerosols are not formed, on account of the physico-chemical properties of the substance, inhalation exposure to vapour is assumed to be negligible.

Immediate skin contact with the diluted solution will occur often, if no PPE is used. Contamination with concentrated solutions will happen more seldom (nearly 1-2 times per day for a few minutes), when concentrated cleanser are manually applied during transfer for dilution or cleaning of very dirty objects.

Inhalation exposure

Workplace measurements

No workplace measurements relating to trisodium nitrilotriacetate are available. In the following, an estimation of exposure levels is performed by analogy.

The Federal Institute for Occupational Safety and Health (BAuA, Germany) conducted workplace measurements during high-pressure cleaning of cars in a washing bay (worst case: closed room, ventilation only during the exchange of cars). A component of the cleaning agent, linear alkyl benzene sulphonate (LAS, concentration in the cleaning product about 0.7 %) was detected using ion chromatography. From 20 measurement results (sampling duration 15 - 210 min) 18 were below the detection limit (0.17 mg/m³, sampling time 210 min). Two results amount to 0.18 mg/m³ and 0.3 mg/m³. The analogy of LAS and trisodium nitrilotriacetate is justified because both substances are solid at room temperature and both are used in low concentrations in cleaning agents. As a rough estimation an exposure level for trisodium nitrilotriacetate can be derived if the different concentrations of LAS (0.7 %) and trisodium nitrilotriacetate (2 %) in cleaning products are considered. This leads to exposure levels of trisodium nitrilotriacetate of ca. 0.6 and 0.9 mg/m³. Taking into account that most of the measurement results are lower than the detection limit and that the high-pressure cleaning was performed in a room without permanent ventilation, the lower value of 0.6 mg/m³ is regarded to represent a reasonable worst case. A request at petrol stations and car washing premises revealed that in Germany high-pressure cleaning is not carried out during the whole shift, but irregularly and only for few hours. For the assessment of an 8h-shift average, a daily duration of 4h/day is assumed. This leads to a shift average of 0.3 mg/m³.

EASE estimation

The EASE model is not applicable to this scenario.

Conclusions

For the assessment of risks of inhalation exposure to trisodium nitrilotriacetate during the high-pressure cleaning 0.3 mg/m^3 (determined by analogy) should be taken as an 8h-shift average and 0.6 mg/m^3 should be taken as an exposure level for the duration of spray-cleaning. It is assumed, that the estimated values can be regarded to represent a reasonable worst case. For the other sections exposure levels are assumed to be lower.

Exposure to dust during the use of powdery formulations is in the same range or lower, because the concentration of trisodium nitrilotriacetate is very low ($< 1 \%$).

Dermal exposure

At the use of products which contains trisodium nitrilotriacetate, here high-pressure cleanser and all-purpose cleanser, dermal exposure is to be considered.

It cannot be excluded, that preparations and products containing trisodium nitrilotriacetate are handled without using suitable personal protective equipment (here gloves and eye protection) (Kliemt 1995). However, in spite of this, dermal exposure may occur due to contamination during the handling of the high-pressure cleaning. Since no measurement results are available, an attempt is made to quantify dermal exposure for the above mentioned situation in application of the EASE model. Taking into account that intermediate dermal contact may occur, however, this situation could be described by the scenario:

Input parameters: wide dispersive use, direct handling, extensive

Level of exposure: 5 – 15 $\text{mg/cm}^2/\text{day}$.

Dermal exposure during use of high-pressure cleanser

Considering an exposed area of 840 cm^2 (hands fronts and backs) the model yields an exposure level of 4200 – 12600 mg/person/day . Taking into account that the concentration of trisodium nitrilotriacetate is 2 % the dermal exposure of 84 – 252 mg/person/day is calculated.

Dermal exposure during use of cleansers (e.g. all-purpose cleanser)

During dermal repeated exposure the estimation according the EASE model (extensive contact and wide dispersive use) yields 5 – 15 $\text{mg/cm}^2/\text{day}$. With regard to the concentration of 0.15 % (diluted cleaning solutions) and an exposed area of 840 cm^2 (hands fronts and backs) an exposure level of 6.3 – 19 mg/person/day is used. The use of concentrated solutions (15 % trisodiumnitrilotriacetate) occur incidental ($0.1 \text{ mg/cm}^2/\text{day}$, exposed area 420 cm^2) and leads to an exposure level of 6.3 mg/person/day .

4.1.1.2.4 Summary

Trisodium nitrilotriacetate is produced /or imported as a liquid and as powder by several companies in the EU. The consumption of trisodium nitrilotriacetate is widely-used.

Three scenarios were established:

Scenario 1: Production of trisodium nitrilotriacetate

Scenario 2: Use of trisodium nitrilotriacetate in formulation processes

Scenario 3: Uses of formulations.

Measurement values regarding the production of trisodium nitrilotriacetate as liquid and as powder are provided by two companies. The production is realized in a closed system. Therefore the inhalation exposure is relevant for drumming of trisodium nitrilotriacetate powder.

For handling the pure substance the use of suitable gloves is considered leading to reduced dermal exposure.

Scenario 2 describes the handling of powder trisodium nitrilotriacetate and also of a 40 % aqueous trisodium nitrilotriacetate solution. Filling, dumping, mixing and weighing activities are to be considered.

Because only a few measurement data are available, an estimation of the inhalation exposure is undertaken using the EASE model.

For the dermal exposure estimation two situations are considered. First is dumping of powdery trisodium nitrilotriacetate and second is handling of a 40 % liquid trisodium nitrilotriacetate solution. The assessment of exposure level is performed by analogy.

The dermal exposure is assessed for the unprotected worker.

Referenced to the third scenario inhalation exposure has to be considered for the formation of aerosols during the application of high-pressure cleaning. The assessment of exposure level is performed by analogy.

The dermal exposure is assessed quantitatively for the high-pressure application by EASE for the unprotected worker.

Furthermore in scenario 3 the professional use of cleansers (all-purpose cleanser) is considered. The available information indicates that this product types are not used by spraying. At workplaces where aerosols are not formed, on account of the physico-chemical properties of trisodium nitrilotriacetate, inhalation exposure to vapour is assumed to be negligible.

Immediate skin contact with the diluted solution of cleansers will occur often, if no PPE is used. Contamination with concentrated solutions will happen more seldom. The dermal exposure is assessed by EASE for the unprotected worker.

4.1.1.2.4 A: Summary of inhalation exposure data (reasonable worst case) of trisodiumnitrotriacetate (Na₃NTA) which are relevant for occupational risk assessment

Inhalation Exposure								
Scenario number, Area of production and use	Form of exposure	Activity	Duration [h/days]	Frequency [days/year]	Shift average concentration [mg/m ³]	Method	Short-term concentration [mg/m ³]	Method
Production								
1) Production of Na ₃ NTA	dust ⁽¹⁾	sampling, cleaning/	daily	daily	3.9	Workplace-measurements (95 th -percentile)	-	-
Use of formulations								
2) Use of Na ₃ NTA in formulation processes	dust	dumping, filling, mixing	1 h	daily	0.62	EASE (with LEV)	-	-
	dust		1 h	daily	6.25	EASE (without LEV)	-	-
3) High pressure cleaning (diluted solutions, < 2 % NTA)	droplet aerosols	spraying	4 hours (assumed)	Daily	0.3 ⁽²⁾	determined by analogy	0.6	determined by analogy

⁽¹⁾ Due to the physico-chemical properties (solid at room temperature, low vapour pressure (< 1 Pa)), inhalation exposures to vapour during the handling of solutions to be negligible.

⁽²⁾ Exposure assessment exemplary for all uses of formulations (liquid, powdery) where the formation of aerosols are probable. Exposure is negligible, if aerosols are not formed.

4.1.1.2.4 B: Summary of dermal exposure data (reasonable worst case) of trisodiumnitrotriacetate (Na₃NTA) which are relevant for occupational risk assessment

Dermal Exposure								
Scenario number, Area of production and use	Form of exposure	Activity	Frequency [days/year]	Contact level	Level of exposure [mg/cm ² /day]	Exposed area [cm ²]	Shift average concentration [mg/person/day]	Method (use of gloves)
Production								
1) Production of Na ₃ NTA	dust	sack-filling, bagging,	daily (assumed)	intermittent	1	420	42	EASE (use of suitable gloves)
	liquid	drumming	daily (assumed)	intermittent	1	210	8.4	
Use of formulations								
2) Use of Na ₃ NTA in formulation processes	dust	dumping,	daily	-	1.9	-	3000 ¹⁾	determined by analogy (without gloves)
	liquid	weighing, filling,	daily	-	14.0	-	4600 ²⁾	
3) High pressure cleaning (diluted solutions, < 2 %)	droplet aerosols	spraying	4 hours (assumed)	extensive	15	840	252	EASE (without gloves)
Use of cleansers (all-purpose cleanser, < 0.15 %)	liquid	wiping	daily	extensive	15	840	19 ³⁾	EASE (without gloves)

- 1) For loading of mixers with powders a typical value of 900 mg/person/day are given.
- 2) For loading of mixers with liquids (40 % trisodium nitrilotriacetate) a typical value of 164 mg/person/day are given.
- 3) Incidental use of concentrated solutions (15 %) leads to a dermal exposure of 6.3 mg/person/day.

4.1.1.3 Consumer exposure

Because the information of the Swedish product register and the BfR data base for consumer products have not been updated, data from the German Federal Environmental Agency (UBA) are used to assess consumer exposure.

According to the UBA information data base required by article 9 of the washing and cleansing agent act (UBA, Dec. 2006), Na₃NTA is used in all-purpose cleaners (up to 15 %), oven cleaners (up to 6 %), floor cleaners and polish (up to 20 %), machine dishwashing products (up to 7.5 %), glass cleaners (up to 8 %), bathroom cleaners (up to 7.5 %), hand dishwashing products (up to 0.8 %), laundry products (up to 3 %), and carpet cleaners (up to 3.5 %).

Furthermore Na₃NTA is contained in car care products. Despite the high concentration in auto insect removers (30%) the inhalation and dermal exposure is negligible, because this product is a ready to use spray, and the frequency and duration of exposure are limited. Therefore these products are not included in the assessment of the consumer exposure.

Inhalation exposure

Exposure to dust from dishwashing powder

Loading a dish washing machine with powder may lead to release of dust and could potentially result in the inhalation of these dust particles.

Van de Plassche et al. (1998) determined an average release of approx. 0.27 µg dust per cup (200 gram) of product used for one stage of washing programme. For granules, it is assumed that a maximum of 10 % is present in the form of powder (Cleaning Product fact sheet, 2006) and therefore the inhalation exposure is expected to be 10-fold lower.

Taking the worst case assumption that all released dust is inhaled, the concentration of Na₃NTA in dishwashing powder is 7.5 % (information from UBA), and the frequency of use is once per day,

the exposure to NTA Na₃ of an adult with a body weight of 60 kg is estimated as following:

$$\text{Expo}_{\text{external}} = (0.27 \mu\text{g}) \times 0.075 \times 1 / 60 \text{ kg (HERA Alcohol Sulphates, draft 2006).}$$

Accordingly the external exposure accounts for **3.4 x 10⁻⁴ µg/kg bw/event**.

With repeated application per day this value is to be multiplied by the appropriate frequency. The estimated above-mentioned value shows clearly that the inhalation exposure by loading a dish washing machine with powder is considered to be negligible.

Exposure to aerosol from cleaning spray

Na₃ NTA is also present in cleaning sprays with concentrations up to 8 %.

The applications of these products take place in 2 phases. In phase 1 the product is sprayed on the surface and in phase 2 the sprayed surface is wiped off and/or cleaned. For this purpose a dry towel or a sponge is used.

During spraying, inhalation can occur and the droplets of the product can be inhaled.

Examples are cleaning sprays such as bathroom cleaner, all-purpose cleaner, oven cleaner and glass cleaner.

To calculate the inhalation exposure, for the above-mentioned sprays the “spray model” from Consexpo 4.1 is used.

Consexpo 4.1 defaults and the results are represented in table 1. These defaults are explained in detail in the “Cleaning Products Fact Sheet” of the RIVM (Prud'homme de Lodder LCH, 2006).

Table 4.1.1.3A: The input variables for model estimations taking the CONSEXPO-software 4.1 and results of inhalation exposure (mean event concentration) to four different cleaning sprays

	all-purpose cleaner	oven cleaner	bathroom cleaner	glass cleaner	dimension
spray duration	0.41	0.5	1.5	0.7	min
exposure duration	60	60	25	240	min
room volume	15	15	10	58	m ³
room height	2.5	2.5	2.5	2.5	m
ventilation rate	2.5	2.5	2	0.5	1/h
mass generation rate	0.78	0.78	0.39	0.78	g/sec
airborne fraction	0.2	0.2	0.2	0.2	fraction
weight fraction non-volatile *	1.4	1	7.5	8	%
density non-volatile **	1.77	1.77	1.77	1.77	g/cm ³
initial particle distribution median (C.V.)	0.6	0.6	0.6	0.6	fraction
inhalation cut-off diameter	15	15	16	15	µm
mean event conc.	0.00007	0.00006	2.7	0.00006	µg/m ³

* These values are product specific

** These values are substance specific

Exposure to aerosol from pressure washers

Pressure washers (high- pressure sprayers) are also used within the private sector. For example these washers are applied for cleaning terraces, cars or the outside of the house. Therefore consumer exposure is possible. This work usually takes place outdoors. A scenario from the RIVM report “Cleaning Products Fact Sheet” (Prud’homme de Lodder LCH 2006) describes the cleaning of a terrace. In this report the indicative surrogate value for inhalation exposure is calculated with 198 mg spray liquid per m³ for application inclusive mixing and loading. If the value given above is taken and the concentration of Na₃ NTA in spray liquid account for 2 % the external inhalation exposure is calculated with 3.96 mg Na₃ NTA per m³ (0.02 x 198 = 3.96).

The BAuA (Germany) conducted workplace measurements during high-pressure cleaning of cars in a washing bay (worst case: closed room, ventilation only during the exchange of cars; see section 4.1.1.2.3). On the basis of these measurements an exposure level of 0.6 mg/m³ and 0.9 mg/m³ was derived for Na₃ NTA considering a concentration of 2 % in the cleaning product. Taking into account that the high-pressure cleaning was performed in a room without ventilation, the value of 0.6 mg/m³ presents the worst case.

The value of 3.96 mg Na₃ NTA per m³ calculated according to the RIVM defaults seems to be a strong over estimation compared to the much lower values derived by the BAuA.

Dermal exposure

Exposure to liquid cleaner and floor polish

Dermal exposure with Na₃ NTA is possible during cleaning processes in the household.

By the example of an all-purpose cleaner the used approach is described:

It is assumed that both hands may come into contact with aqueous solution containing the cleaner. The concentration of substance in product before dilution amounts to 15 % (worst case).

In the literature different dilution factors for liquid cleaners are described. Prud’homme de Lodder et al. (2006) proposes 80 and the Final Report Ecolabel for Cleaners (2000) 125. Therefore a dilution factor of 100 is assumed.

Taking the formula

$$A_{\text{der}} = C_{\text{prod}}/D * TH_{\text{der}} * \text{Area}_{\text{der}} \text{ (compares TGD)}$$

the amount of substance on skin per event can be calculated. The abbreviations are explained below.

If the following values are used

$$C_{\text{prod}} = 150 \text{ mg/cm}^3 \text{ (=15 \%)}$$

$$D = 100$$

$$TH_{\text{der}} = 0.01 \text{ cm}$$

$$Area_{\text{der}} = 840 \text{ cm}^2$$

the external dermal exposure (amount of substance on skin per event) accounts for 12.6 mg per event.

The potential uptake per kilogram body weight (60 kg) per day is derived as:

$$U_{\text{der, pot.}} = A_{\text{der}}/bw * n$$

If the mean number of events per day for all purpose cleaners is set 0.28 (104 events/year) the amount of Na₃ NTA that can potentially be taken up per day accounts for 0.059 mg/kg bw/d.

The input variables taken for estimation and the results are shown in table 4.1.1.3B.

Table 4.1.1.3B: Potential external dermal exposure to cleaner and floor polish (diluted and undiluted)

	all-purpose cleaner	floor cleaning liquid	hand dishwashing liquid	detergent powder - hand wash	floor polish liquid (undiluted)	oven cleaner liquid (undiluted)	dimension
C _{product}	15	20	0.8	3	20	6	%
C _{product}	150	200	8.0	30.0	200	60.0	mg/cm ³
D	100	100	714 *	100*	1	1	
C _{der}	1.5	2	0.011	0.30	200	60	mg/cm ³
TH _{der}	0.01	0.01	0.01	0.01	0.01	0.01	cm
AREA _{der}	840	840	840	840	420	420	cm ²
V _{appl}	8.4	8.4	8.4	8.4	4.2	4.2	cm ³
bw	60	60	60	60	60	60	kg
frequency (RIVM default)	104	104	426	104	2	26	1/year
n (per day=d ⁻¹)	0.28	0.28	1.17	0.28	0.005	0.071	
A _{der} (C _{der} * V _{appl})	12.6	16.8	0.094	2.5	840	252	mg/per event

U_{der.pot} (A_{der} / bw)	0.210	0.280	0.002	0.042	14.0	4.2	mg/kg bw/event
U_{der.pot} (A_{der} /bw * n)	0.059	0.078	0.002	0.012	0.070	0.298	mg/kg bw/day

* RIVM default

Explanation of abbreviations:

C _{product}	concentration of substance in product before dilution
D	dilution factor
C _{der}	dermal concentration of substance on skin
TH _{der}	thickness of product layer on skin
AREA _{der}	area of contact between product and skin
V _{appl}	volume of diluted product actually contacting the skin
bw	body weight
n (per day=d ⁻¹)	mean number of events per day
A _{der} (C _{der} * V _{appl})	amount of substance on skin per event
U _{der.pot} (A _{der} / bw)	amount of substance that can potentially be taken up per event
U _{der.pot} (A _{der} / bw * n)	amount of substance that can potentially be taken up per day

Exposure from spray cleaner

To calculate the dermal exposure after spraying, the Consexpo 4.1 is used for phase 2 application: cleaning.

The input variables, which are explained in detail in the fact sheet of the RIVM (Prud'homme de Lodder LCH, 2006), taken for estimation and the results are represented in table 4.1.1.3.

Table 4.1.1.3C: The input variables for model estimations taking the CONSEXPO-software 4.1 and results of external dermal exposure to four different cleaning sprays

	all-purpose cleaner	oven cleaner	glass cleaner	bathroom cleaner	dimension
exposed area	215	430	215	215	cm ²
product amount	0.16	0.2	0.29	0.3	gram
exposure duration	3.2	20	6	20	min
weight fraction compound	1.4	1	8	7.5	%
body weight	60	60	60	60	kg
exposure frequency	365	26	365	52	1/year
external exposure (50th percentiles) per event	0.037	0.033	0.387	0.375	mg/kg bw/event
external exposure (50th percentiles) per day	0.037	0.002	0.387	0.053	mg/kg bw/day

Exposure from pressure washers

Dermal exposure may occur due to contamination during the handling of the high-pressure washer without using gloves.

According to the report “Cleaning Products Fact Sheet” (2006) an indicative surrogate value for dermal exposure is derived (details see there). This value accounts for 2100 mg spray liquid per minute.

The dermal exposure is calculated as spray liquid/min x concentration in spray liquid x application duration (min/event) which gives an exposure of 2100 x 0.02 x 30 = 1260 mg Na₃ NTA and referring to a body weight of 60 kg amounts to 21 mg/kg bw/per event. The frequency of spraying is once a month and therefore the mean number of events per day is 0.033.

The potential uptake per kg bw (60 kg) per day accounts for 0.69 mg/kg bw/day (1260 x 0.033/ 60).

For this scenario the equation of the TGD can also be used. The amount of Na₃ NTA on skin is calculated as described in chapter “Dermal exposure, liquid cleaners”.

Considering an exposed dermal area of 840 cm² (hand fronts and backs), a concentration of trisodium nitrilotriacetate (2 %) in the spray liquid (diluted product) and the thickness of spray liquid layer on skin 0.01 cm the calculation yields an external dermal exposure of 168 mg per event and referring to a body weight of 60 kg amounts to 2.8 mg/kg bw/per event. The amount of Na₃ NTA that can potentially be taken up per kilogram body weight (60 kg) per day accounts for 0.092 mg/kg bw/d (168 x 0.033/60). This value is 7.5-fold lower

compared to the value of 0.69 mg/kg bw/day calculated according to the report "Cleaning Products Fact Sheet" (2006).

Exposure from residues on textile/clothing

After treatment of fabrics/textiles with Na₃ NTA solution residues may remain on fabrics/textiles. When clothing - manufactured of this fabrics/textiles - contacting the skin, residues can migrate from textile to skin.

According to measurements in textiles by BASF Na₃ NTA residues can reach up to ~ 1.0 mg/kg of textiles. Assuming, that 1 m² will be covered by 100 g of the textile 100 µg could migrate during first wearing and referring to the body weight of 60 kg an external potential dermal exposure of 1.7 µg/kg bw can be calculated.

Oral exposure

Exposure from residues on dinnerware

Oral exposure can occur by use of dish-washing detergents containing Na₃ NTA.

The quantity of residues increases with the detergent concentration and not drying the dinnerware results in a higher amount of Na₃ NTA on the dishes and glassware.

According to RIVM (Prud'homme de Lodder LCH, 2006) a "hand-wash" scenario is assumed, in which all dinnerware is not dried with a dishcloth.

The value for amount of water left on dishes is 5.5×10^{-5} ml/cm², the value for the area of dishes in daily contact with food is 5400 cm² and the concentration of the product in dishwashing water is 1.4 mg/cm³. Using these data, the possible ingested product amount is

$$5.5 \times 10^{-5} \text{ ml/cm}^2 \times 5400 \text{ cm}^2 \times 1.4 \text{ mg/ml} = 0.4158 \text{ mg}$$

If the frequency is once a day and the concentration of Na₃ NTA in the product is 0.8 % an amount of 3.33 µg could contaminate food and could be ingested daily. Related to a body weight of 60 kg the potential oral exposure can amount to 0.055 µg/kg bw/day.

Conclusion

Inhalation exposure

The inhalation exposure by loading a dish washing machine with powder accounts for

3.4×10^{-4} µg/kg bw/event and can be neglected. Furthermore surface spray can lead to inhalation exposure. However, only when bath room cleaner is sprayed an exposure level

(mean event concentration) of $2.7 \mu\text{g}/\text{m}^3$ can be reached during 20 minutes and once a week (Consexpo 4.1). Due to low volatility, together with low use frequency (once per week) we don't assume chronic exposure. The estimated exposure levels for the other spray applications are considered to be negligible. The inhalation exposure to aerosol has also been considered during application of high-pressure cleaning. The assessment of exposure level is performed by analogy to the occupational exposure, scenario 3. An exposure level of $0.6 \text{ mg}/\text{m}^3$ represents the worst case during 30 minutes and once a month.

Dermal exposure

Dermal exposure to Na_3 NTA can occur from application of different cleaners and from residues on textiles during first wearing.

Exposure from cleaners

Three scenarios for cleaners were established.

1. Exposure to liquid cleaner and floor polish

Taking worst case assumptions the highest values of dermal exposure are reached by use of floor polish liquid (undiluted) per event = $14 \text{ mg}/\text{kg}$ bw and by use of oven cleaner liquid (undiluted) per event = $4.2 \text{ mg}/\text{kg}$ bw (TGD). The other values of all-purpose cleaner, floor cleaning liquid, hand dish-washing liquid and detergent powder-hand wash were 1 to 2 orders of magnitude lower. Considering the frequency per year the dermal exposure per day has been estimated. Aggregated dermal exposure accounts for $0.519 \text{ mg}/\text{kg}$ bw/day when all applications take place on one day.

2. Exposure from spray cleaner

For the assessment of dermal exposure caused by spray cleaners, only glass and bath room cleaners are important. The estimated values (50. percentile, Consexpo 4.1) per event of all-purpose cleaners and oven cleaners are lower than those use of liquid cleaners. Aggregated dermal exposure accounts for $0.440 \text{ mg}/\text{kg}$ bw/day when glass and bath room cleaners have been applied on one day.

3. Exposure from pressure washers

External dermal exposure amounts 168 mg per event (TGD) or $2.8 \text{ mg}/\text{kg}$ bw/per event (referring to a body weight of 60 kg).

According to a frequency of use per day of 0.033 (once a month) the amount of Na_3 NTA that can potentially be taken up per kilogram body weight (60 kg) per day accounts for $0.092 \text{ mg}/\text{kg}$ bw/d.

Exposure from residues on textile/clothing

Residues of Na₃ NTA may remain on fabrics/textiles after manufacturing and can migrate during first wearing from textile to skin.

Taking into consideration the measurements by BASF an external potential dermal exposure of 1.7 µg/kg bw can be calculated for first wearing of clothing.

Oral exposure

Residues of detergents containing Na₃ NTA on dinnerware can contaminate food and can be ingested. According to the Cleaning Products Fact Sheet (Cleaning Products Fact Sheet, RIVM, 2006) the amount Na₃ NTA which represents the daily potential oral uptake accounts for 0.055 µg/kg bw/day.

4.1.1.4 Indirect exposure via the environment

As NTA does not accumulate in biota, a significant intake via fish, plants or meat is not expected. The predominant exposure path for human is the uptake via drinking water.

Production and treatment of drinking water

The behaviour of EDTA at different drinking water purification techniques was studied in several investigations.

NTA was measured in river water (Elbe, Germany) and bank filtrate at a site where due to a high pollution with organic substances the filtration zone was in an anaerobic state. At this site the retention time in the aquifer is only a few days. The NTA concentration (50%iles) decreased from 3.5 µg/l in the river water to <0.5 µg/l in the bank filtrate (Guderitz et al., 1993).

Similar results were observed in the Swiss river Glatt: the NTA concentration of 5 – 55 µg/l in the river water decreased in the groundwater to 0.3 – 1.3 µg/l at a filtration distance of 2.5 m. At a distance of 14 m the concentration was below the detection limit of 0.2 µg/l (Giger, 1984).

NTA degradation and mobilisation of heavy metals during river bank filtration was investigated in a laboratory experiment. Columns (length 1.2 m; diameter 0.18 m) were filled with river bottom sludge collected from Merwede (Netherlands) and percolated with river water containing up to 600 µg/l NTA (unclear whether acid or Na-salt). In the input river water, which was renewed bi-weekly, NTA was completely degraded within two weeks, both in O₂- containing and –deficient water. Total contents of Cu, Zn, and Ni in the sludge were 130, 1600, and 130 mg/kg respectively. Cu, Zn, and Cd in the percolate were reduced by 50% compared to the river water, while Ni and Pb were not reduced (Loch et al., 1983).

Schick (1994) summarised the removal of NTA during several steps of drinking water purification: In a test filter system simulating the removal during bank filtration, 20 µg/l NTA

degraded with a half-life of about 4 days. At a water work at the river Rhine with a filtration distance of 20 m, NTA was nearly completely removed. In a test apparatus for slow sand filtration, NTA was completely removed at a distance of 70 – 100 cm after an adoption period of about 3 weeks. NTA does not react with chlorine or chlorine dioxide, while with ozone treatment a large part is removed. Filtration with charcoal leads to a low removal because of the poor adsorption of NTA.

In drinking water produced by bank filtration from the river Ruhr, NTA was not detected (<1 µg/l), while in the river water up to 9.1 µg/l were detected (AWWR, 2000).

Model calculation

Based on the physico-chemical properties of NTA (logKow <4; Henry's law constant <100 Pa.m³.mol⁻¹) and the aerobic biodegradation rate (<10 d), a purification factor of ¼ is proposed by the TGDs. The studies referred above reveal that the removal during bank filtration is higher than the TGD default value. The following exposure calculations, a purification of 90% is assumed, all further parameters are taken from the TGDs.

Table 4.1.1.4.A: Calculation of the indirect exposure of human via the environment

Scenario	PEC _{local} _{aqua} [µg/l]	DOSE _{tot} [mg.kg bw ⁻¹ .d ⁻¹]
Producer B	< 5.1	4.0 E-5
Producer C	5.6	4.4 E-5
Producer D	450	3.5 E-3
Producer E	14	1.1 E-4
Textile Cleaning, Formulation	20	1.6 E-4
Textile Cleaning, Use	500	3.9 E-3
Cleaning agents, Formulation	49	3.8 E-4
Cleaning agents, Use	49	3.8 E-4
Regional Scenario	4.2	3.3 E-5

In all scenarios, 37% of the intake is due to drinking water and 63% to fish. Other pathways are not important.

4.1.1.5 (Combined exposure)

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

Available animal data have been obtained after oral, iv and ip application of NTA. There are not data from inhalative or dermal exposure.

Oral data

In an absorption and excretion study conducted by the BASF AG (unpublished report, 1997) non-fasted Wistar rats were administered by gavage a single high dose (441 mg/kg bw; four male animals) and a single low dose of Na₃NTA x H₂O (22 mg/kg bw; four male animals). The substance was given as cold (i.e. non-radiolabelled) substance. Furthermore, four male animals were dosed daily with 453 mg/kg bw (nominal dose: 500 mg/kg bw) Na₃NTA x H₂O for 7 consecutive days. Urine was collected 6, 12, 24, 48 and 72 hr after the single administration and in 24-hr intervals after repeated dosing. The analysis of NTA in the application solutions and urinary samples was performed by HPLC. Feces, carcass and expired air have not been analyzed and the recovery in the various experiments has not been provided. Excretion of NTA in the urine was rapid for single and repeated dosing with an urinary excretion half-life of about 5 to 6 hr. However, the absorption (as measured by urinary excretion of NTA) was in all three applications incomplete amounting to about 50% of the dose applied.

Michael and Wakim (1971) have studied absorption, distribution and metabolism of ¹⁴C-labeled NTA (4.6 mCi/g, radiolabel in the carboxyl groups of NTA, 100 % purity) as an aqueous solution containing disodium salt Na₂NTA after single and repeated dose in male Sprague-Dawley rats and after single oral doses in a male New Zealand rabbit, a male beagle dog and a female Rhesus monkey.

The distribution and excretion of ¹⁴C-NTA was investigated in groups of 3 rats, respectively, 1, 6, 12, 24, 48, 72 and 168 hours after oral administration of a single dose of 10 mg of an aqueous solution of ¹⁴C-NTA (which corresponds to 40 – 50 mg/kg bw, because rat weights were between 200 and 250 g). NTA was well absorbed in rats, as evidenced by the low recoveries of ¹⁴C in gastrointestinal tract contents only 1 h after p.o. administration of ¹⁴C-NTA. The absorbed ¹⁴C-NTA was rapidly excreted as unchanged substance (detected by the use of isotope dilution techniques and paper chromatography) in the urine: within 1 h, 31% of the dose was found in the urine. An appreciable quantity of ¹⁴C was found in kidney, muscle, blood, and tibia 1 h after administration. During the first 48 h after dosing the concentration of ¹⁴C in the blood and the soft tissues decreased to about 0.04 to 0.06 % of the administered dose. The biological half life of ¹⁴C-NTA in these tissues was approximately 3 h. The radioactivity in the skeleton during the first 48 h after dosing decreased from 9 % to 2 % of the administered ¹⁴C-NTA. Most of the applied amounts of ¹⁴C-NTA were excreted within 72 hours (95 % in urine, 2 % in feces).

After repeated oral doses of ¹⁴C-NTA to rats (10 mg/day for five consecutive days, 2-3 rats per timepoint investigated, time points investigated: 1 hr, 3.5 days, 7 days, 14 days, 21 days, 28 days after end of dosage), the rate of disappearance of ¹⁴C-NTA from the carcass was comparable to the results from single application. For example, the concentration of ¹⁴C in the kidneys decreased to <0.01 % of the administered dose after the 4th day. However, the amount of ¹⁴C-NTA excreted in the feces and

the amount of ^{14}C -NTA remaining in the bone (tibia) was higher after multiple dosing. Lower amounts compared to single dose were excreted via urine (about 59 % of the dose).

Excretion of ^{14}C -NTA was determined 72 hours after single oral administration of 50 mg/kg bw ^{14}C -NTA to a rabbit, a dog and a monkey. Large species differences could be observed concerning urinary and fecal excretion: 23 %, 69 % and 14 % of the administered dose was excreted via urine in the rabbit, dog and monkey, respectively. Amounts excreted via feces were 33 % (rabbit), 5 % (dog) and 65 % (monkey). The absorption and distribution pattern in the dog was similar to that in rat. The exceptionally high amount of ^{14}C -NTA that were detected in the gastrointestinal tract of the rabbit (26 % compared to 0.07 % in the dog and 0.1 % in the monkey) point to the fact, that NTA as absorbed more slowly in the rabbit compared to other species.

Absorption, distribution and metabolism of a single oral dose of 20 mg/kg bw ^{14}C - Na_2NTA (16 $\mu\text{Ci/g}$) in 7 female beagle dogs were studied by Budny (1972). The absorption of ^{14}C -NTA was rapid as indicated by the concentration peak in the blood which occurred around 75 min post treatment. ^{14}C -NTA appearing in the blood was associated with the serum, not the cells. 72 h after po intubation of ^{14}C - Na_2NTA , 3 % of the dose was recovered in the feces, while 80 % appeared in the urine. No metabolites were observed in either urine or feces by the use of reverse isotope dilution and thin layer chromatography.

Greatest tissue deposition is seen in bone (2-3 $\mu\text{g Na}_2\text{NTA/g}$), followed by the kidney. Other organs analysed (leg and jaw muscle, stomach-esophagus, intestine, heart, lung, liver, spleen, brain, pancreas, adrenal, ovary and urinary bladder) contained <0.3 $\mu\text{g/g}$ tissue.

Distribution, metabolism and excretion of a single oral dose of 180 mg/kg bw (40 μCi) ^{14}C -NTA (H_3NTA , dissolved in phosphate buffer carboxyl- labelled, 58 mCi/mmol) was investigated in non-fasted male albino mice (Chu et al., 1978). In five animals, serial blood samples were taken from the tail at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h after administration. After the last sampling, animals were exsanguinated and tissues (liver, brain, heart, kidney, lung, spleen, bladder, testes, a portion of the femur, muscle, skin and fat) were taken for analysis of radioactivity. Furthermore, groups of five animals were exsanguinated at 1, 8, 24 and 48 hr after administration and tissues were collected for estimation of radioactivity. Five further animals were individually kept in metabolism cages and urine and feces were collected daily for seven days. Radioactivity in blood, tissues and excreta was determined by liquid scintillation. Identification of radioactivity in urine and feces was performed by thin layer chromatography (TLC) and Gas-chromatography/mass spectrometry (GC/MS). Blood concentration of ^{14}C -NTA peaked 1 h after administration. At 4 h about 85 % of the radioactivity was removed from the blood. ^{14}C -NTA was rapidly distributed at 1 h in the liver, brain, heart, lung, spleen, testes, a portion of the femur, muscle, skin and fat. Higher concentrations compared other tissues were shown in the bladder, bone and kidney. Approximately 99 % of the dose was eliminated within 24 h, 96 % with the urine and 3 % in the feces. TLC and GC/MS analysis revealed that the single radioactive band found in TLC plates corresponded to unmetabolized NTA.

Weanling male rats (10 animal per substance) were fed various forms of nitrilotriacetate (1.5 % H_3NTA or 2 % Na_3NTA)x H_2O in the feed for 35 days (Anderson and Kanerva, 1978). Urine was collected during the fourth week. For both substances the concentration of NTA in the urine was about 40 % of the dose. The urinary Zn excretions were elevated when both 1.5 % H_3NTA (3.3 $\mu\text{mol Zn}/100 \text{ g bw/d}$ vs. 0.2 $\mu\text{mol Zn}/100 \text{ g bw/d}$ in controls) or 2 % Na_3NTA x H_2O (3.0 $\mu\text{mol Zn}/100 \text{ g bw/d}$ vs. 0.2 $\mu\text{mol Zn}/100 \text{ g bw/d}$ in controls) were fed. The disposition of Cu, Fe and Mn was not appreciably influenced by NTA administration.

Groups of seven female Fischer 344 and Charles River CD rats were given, for 42 and 30 days respectively, NTA as $\text{Na}_3\text{NTA} \times \text{H}_2\text{O}$ at dietary levels of 0.05 to 2 %. NTA did not accumulate in the bladder tissues to any greater extent than in the heart and liver, even when urinary NTA levels were up to two hundred times higher than those in the plasma. A small amount of insoluble NTA (as CaNaNTA , approximately 7 % of the total urinary NTA) was present in the urine of rats of both strains fed 0.05 or 0.1 % NTA. At greater dietary NTA levels, when urinary NTA concentration exceeded 3 $\mu\text{mol/ml}$, there was a linear increase in the amount of insoluble NTA. The crystalluria occurs when urinary NTA exceeds the load produced by a dietary level of 0.3 % $\text{Na}_3\text{NTA} \times \text{H}_2\text{O}$ (Anderson, 1980).

Other routes

In order to investigate the route of absorption and the possibility of enterohepatic circulation, Michael and Wakim (1971) compared distribution and excretion of ^{14}C -NTA among unoperated rats (4 animals, po intubation of 10 mg ^{14}C -NTA), bile-duct cannulated rats (6 animals, ip intubation of 10 mg ^{14}C -NTA) and thoracic-duct cannulated rats (po injection of 10 mg ^{14}C -NTA). In unoperated, orally dosed rats, 70 % of the administered ^{14}C -NTA was excreted in the urine and 22 % was excreted in the feces 51 h after administration. Less than 1 % of the administered dose was excreted in the expired CO_2 . No more than 3 % of the administered material was found to remain in the body; most of this was found in the skeleton (8 μg NTA/g bone). The distribution of 10 mg ^{14}C -NTA after i.p. injection into bile duct-cannulated rats was similar to that found in unoperated rats that had received ^{14}C -NTA p.o. These findings confirm that ^{14}C -NTA did not enter the enterohepatic circulation (< 0.12 % of the dose). In addition, by using thoracic duct-cannulated rats (3 animals), it was found that ^{14}C -NTA was not appreciably absorbed and transported by the lymphatic system (< 4 % of the dose).

The renal clearance (C) of Na_2NTA in rats (number of animals not given), which was determined to be 2.33 ml/min, has been compared to that of inulin at NTA iv infusion rates ranging from 2.5 to 20 $\mu\text{mol/kg/h}$ which resulted in plasma concentrations of 3 to 33 $\mu\text{mol/ml}$. Under these conditions the $C_{\text{NTA}}/C_{\text{inulin}}$ ratio was 0.89 and was independent of NTA dose. Furthermore, the C_{NTA} was altered in rats pre-treated with a tubular cell transport poison (probenecid, 200 mg/kg i.v. 24 h before NTA-clearance) or when measured with simultaneous infusion of an organic acid transport competitor (p-aminohippuric acid, 50 mg iv/rat). Under these conditions the C_{NTA} was 2.71 and 2.38 respectively. Thus, the clearance of NTA is accomplished by passive glomerular filtration (Anderson et al., 1985).

Budny et al. (1972) investigated excretion of ^{14}C -NTA after iv administration to male and female beagle dogs (exact number of animals not given). 72 hours after administration of 20 mg/kg to dogs, 96 % of the dose was recovered in the urine and 0.7 % of the dose was detected in the feces (mean of 3 dogs). Therefore the authors concluded that no enterohepatic circulation occurred in the dog.

Chu et al (1978) investigated distribution and excretion of ^{14}C -NTA (H_3NTA , dissolved in phosphate buffer carboxyl- labelled, 58 mCi/mmol) after iv administration to male albino mice. Five mice received 45 mg/kg bw (10 μCi) ^{14}C -NTA and blood was taken in 2 min intervals for 20 min and 30 and 60 min after dosing. Five animals received 45 mg/kg bw (10

μCi) ^{14}C -NTA and were exsanguinated at 1, 8, 24 and 48 hr after administration. Tissues were collected for estimation of radioactivity. Furthermore, 45 mg/kg bw and 4.5 mg/kg bw of ^{14}C -NTA was also administered intravenously to bile-duct cannulated male mice (2 animals per dose). Bile samples were collected hourly for 8 hours. Radioactivity was determined by liquid scintillation. 1 hour after administration, radioactivity could be detected in all tissues investigated. The highest concentrations were observed the bladder, the kidneys and the bone. Radioactivity could not be determined in tissues obtained after 8, 24 and 48 hours. Examination of the biles indicated that little radioactivity (< 1 %) was present. Compared to orally dosed animals, higher concentrations of radioactivity were found in kidneys, heart and skin of iv dosed mice 1 hr after administration.

Female Sprague-Dawley rats (catheterised to collect urine) were given an iv infusion of 5 % mannitol (3.5 ml/hr) to produce an osmotic diuresis within 6 h (Pollack and Ruocco, 1981). During the first hour the urinary iron excretion averaged 0.29 $\mu\text{g/h}$. In subsequent hours urinary iron became lower than the detection limit (< 0.2 $\mu\text{g/h}$). An intraperitoneal Na₂NTA-dose of 77 mg per 250 g rat was given to 8 rats at the end of the third hour of the infusion. Urinary iron remained too low to be detected in the animals despite the administration of NTA. The effect of NTA (77 mg/ml/250 g given i.p. at 40 min) on ^{59}Fe clearance from plasma was determined in two rats. The plasma iron clearance was not affected by NTA.

The effect of 100 mg NTA/kg bw (administered i.p. once daily for 3 consecutive days) on the urinary elimination of several endogenous metals was determined in male Swiss Webster mice (6 animals). The concentrations of Zn, Cu, Mg, Mn, Fe, and Ca were determined in daily urine. Administration of NTA had no significant effect on the urinary elimination of any of the metals studied (Cantilena & Klaasen, 1982).

Four sheep (each 50-55 kg living weight), each prepared with a rumen fistula and re-entrant cannula in the proximal duodenum were used to study the effects of ruminal administration of H₃NTA (0, 300, 600, and 1200 $\mu\text{g/g}$ of diet) on solubilities of zinc, copper, manganese and iron in rumen and duodenal digesta. The water solubilities of the elements in the diet were Zn (82.8 %), Cu (81.5 %), Mn (59.6 %), and Fe (21.6 %). Higher concentrations of soluble zinc, manganese, and iron, but not copper, were found in the rumen of the sheep during the first 2 h after feeding when they were dosed with NTA. The concentrations increased with increasing dose of the acid. However, only the solubility of iron was increased in the duodenal digesta. The concentration of soluble iron in the duodenum during 6 h after feeding increased to 43.7 % (1200 μg NTA/g diet) (Ivan et al., 1978).

Human data

In vivo oral data

10 mg ^{14}C -NTA (9.7 mCi/g, carboxyl-labelled, no further data were given) in gelatine capsules (with 3-4 ounces of fruit juice) was given po to 8 male volunteers after a 10-hr fast. Blood was collected at 0, 15, 30, 45, 60, 75, 90 min and 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h post dosing. Gelatine capsules easily dissolve in aqueous media, so they will have negligible influence on absorption. Urine was collected at each voiding and combined into the following samples: 0-6 h, 6-24 h, and each additional 24 h until completion of the study. Expired CO₂ was collected from 4 of the 8 volunteers (0 to 6 h post dosing). The peak value of ^{14}C -NTA in the blood (6.5 ng/g serum) was reached after approximately 2 h. At 12 h only ^{14}C -NTA

concentrations below 0.1 ng/g serum were observed. 120 h after administration the feces contained 77 % of radioactivity, and 12 % was recovered in the urine and based on findings in animals, it was concluded by the authors that there is little reason to believe that an enterohepatic circulation does occur in man. 87 % of the urinary ^{14}C was excreted in the first 24 h-period. Less than 0.1 % was recovered in the expired air. The total recovery was 89 %. However, the amount of ^{14}C remaining in the tissues was not included in the total recovery figure. Thin-layer chromatography and reverse isotope dilution was applied in order to investigate metabolism of NTA. The results indicated that >96 % of the urinary radioactivity was unchanged NTA (Budny & Arnold, 1973).

In vitro dermal data

Skin penetration of NTA has been investigated in vitro according to OECD 428 and OECD Guidance Document No. 28 by using radiolabeled substance (2- ^{14}C - Na_3NTA), human skin preparations (split thickness skin is prepared human skin (abdomen), thickness around 400 μm) and Franz-type diffusion cells BASF, 2007b). Test substance preparations corresponding to a high (40 % NTA) and low (1% NTA) aqueous dilution were applied for a exposure time of 5 min (it was decided to stop the exposure to the concentrate, which has a high pH, after 5 min (as the preparation of the test substance through largely intact skin preparations should be determined and because “it might be taken as granted that accidental severe skin damage would greatly enhance penetration”) and 6 h (reflecting consumer exposure), respectively. Each preparation (concentration) was applied onto an area of 1.0 cm^2 skin (application dose: 10 $\mu\text{l}/\text{cm}^2$); each concentration was investigated in 5 different skin samples (i.e. 5 different diffusion cells per dose). Diffusion cells were operated in a static mode under semi-occlusive conditions using tap water as receptor medium. Amounts of receptor cell fluid were taken at definite sampling times during a 24h period. At the end of the sampling period, the remaining test substance was recovered from all compartments of the diffusion cell. Radioactivity was determined in different compartments (tape strips, membrane washings, skin preparation, receptor fluid and receptor chamber washings) by liquid scintillation counting.

Results: mean total recoveries of radioactivity were 98.75% (high dose) and 101.8 % (low dose) of the applied radioactivity. The sum of radioactivity present in the skin plus radioactivity determined in the receptor fluid and receptor chamber washing was taken as the absorbed dose.

At the high dose, only very low amounts of substance were absorbed mean: 0.001 % (n=5) of the applied radioactivity). However, the applied dose was washed off after 5 min in this setting, so this experimental setting does not allow to draw conclusions on the skin-penetrating properties of the high dose (40% NTA) dilution.

At the low dose dilution (1% NTA), absorption percentages (percentage in the skin plus percentage in the receptor fluid and chamber washing fluid) from a sampling period of 24 hr ranged between 0.042 and 0.472 % within the five different samples. Thus, due to the high variability in human skin, an absorption rate of 1 % is taken for risk characterisation of consumer exposed to diluted (1 %) NTA solutions (worst case assumption taking into account the high variability in the human samples). For higher NTA concentrations in solution, a higher absorption rate of 10 % (default based on physico-chemical properties due to the lack of meaningful experimental data) is taken for risk characterization.

Conclusion:

NTA, a metal ions chelating agent, is rapidly absorbed in dogs, rats and mice. The biological half-life for the elimination of NTA was approximately three hours. Studies in dogs, rats and mice indicated that NTA did not enter the enterohepatic circulation. The absorbed NTA was rapidly excreted via the urine except for a small fraction remaining in the bone. The urinary clearance is accomplished by passive glomerular filtration in rats. Any biotransformation of NTA was not observed in the studies for men, dogs, rats and mice. Less than one percent of the administered dose was excreted in the expired CO₂. Urinary calcium and zinc concentrations were increased in urine of rats and mice with high urinary NTA levels. The disposition of copper, iron and manganese was not appreciably influenced by oral NTA administration (up to 2 % Na₂NTA or 1.5 % H₃NTA in diet to rats). There are no studies for skin permeation and no information is available on absorption via the inhalation route (aerosol, dust).

Highly variable urinary excretion of NTA has been reported for several species including humans: percentages of the dose excreted via urine were 12 (man), 14 (monkey), 23 (rabbit), 70 (rat), 80 (dog) and 96 (mouse). From experimental findings in bile cannulated animals (mice, rat, dogs) biliary excretion is less than 1%. Thus, it can be assumed that biliary excretion does not play a role with all species taking into account low molecular weight and the absence of specific transporters, although this has not been experimentally determined in humans. Whereas for animals a value of 50% absorption which represents the median of the findings in animals may be appropriate, the findings in human volunteers of 12% urinary excretion without indication that NTA is excreted via bile into the feces point to a lower absorption rate in humans. A value of 20 % absorption is proposed for human risk characterisation. However for the rat, complete (100%) absorption should be taken for risk characterisation.

For 1% NTA dilutions, an absorption rate of 1 % is taken for risk characterisation based on an in vitro dermal absorption study. For higher NTA concentrations in solution, a higher absorption rate of 10 % is taken for risk characterisation based on considerations on the chemical structure and physico-chemical data (molecular weight (257 g/mol), water solubility (660 g/l), partition coefficient (logPow -2.62), and ionisation state of about -2 (pKa 1.9, 2.5 and 9.7) and based on the fact, that a 40 % NTA solution has a high pH, so that skin damage and enhanced penetration due to damaged skin can be anticipated.

Structural (physicochemical) considerations have also to be taken into account for systemic absorption via inhalation. Taking into account that oral absorption is 10-20 % which indicates low permeability through biological membranes, a default value of 20 % is proposed for uptake via inhalation.

4.1.2.2 Acute toxicity

Animal data:

Oral

The data on acute oral toxicity of trisodium nitrilotriacetate currently available demonstrate considerable species differences: For monkeys an approximate LD₅₀ value of 750 mg/kg is

reported, for rats oral LD50 values of 1300-1470 mg/kg body weight for females and 1600-2220 mg/kg bw for males have been detected, and for dogs a LD50 >5000 mg/kg was found.

For adult rhesus monkeys an approximate oral LD50 of 750 mg/kg bw was detected using a 50% aqueous substance suspension of nitrilotriacetic acid trisodium salt (Na₃NTA, no data on purity): One rhesus monkey received 500 mg/kg, 2 rhesus monkeys 1000 mg/kg and 2 further animals 2000 mg/kg of a 50% aqueous substance suspension. The animal treated with 500 mg/kg and 1 animal treated with 1000 mg/kg vomited within 1 minute after dosing. Both animals appeared grossly normal within 10-15 minutes. The animal that received 500 mg/kg was sacrificed after 11 days. No unusual lesions were noted either grossly or microscopically. A second animal receiving 1000 mg/kg died after 70 minutes. This level produced a slight decrease in motor activity 50 minutes after dosing. This was followed by paralysis and then by death. Two animals treated with 2000 mg/kg each died approximately 30 minutes after dosing. A decrease in motor activity was also noted in these animals ca. 15 minutes after dosing, followed in 5 minutes by paralysis and death. The only unusual lesions noted grossly at autopsy were hemorrhagic areas in the stomach. Microscopically, there was also evidence of mild irritation in the stomach (Nixon, 1971).

In a study with nitrilotriacetic acid trisodium salt monohydrate (Na₃NTA x H₂O, purity 99%) according to FIFRA/TSCA Methods of 1982, an oral LD50 of 1300 mg/kg bw for female and of 1600 mg/kg bw for male rats was determined: Single doses of 625, 1250, 2500 and 5000 mg/kg of Na₃NTA in distilled water were administered by oral intubation to 4 groups of 5 male and 5 female rats each. No deaths occurred after treatment with 625 mg/kg, 1 male and 2 females died within 24 hours after administration of 1250 mg/kg, all rats died after treatment with 2500 and 5000 mg/kg within 4 hours. Signs seen on the day of dosing, primarily as antemortem signs in animals which died, included ataxia, tremors, hypopnoea, hypothermia, hypoactivity, prostration and oral, nasal and ocular discharge. A few animals in the 625 and 1250 mg/kg groups exhibited soft stool, unthrifed coats and fecal and/or urinary staining during the 24 hours after dosing. No other abnormalities were seen in the 625 mg/kg group. Several surviving animals in the 1250 mg/kg group had decreased food consumption on the day after dosing, but all surviving animals were free of abnormalities from day 2 through termination of the study on day 14. Postmortem examinations of animals found dead revealed a variety of changes, primarily in the lungs and gastrointestinal tract. Some animals had changes in the stomach and intestine which were suggestive of an irritant effect (red fluid in the stomach and intestine, discoloration of walls), and several had apparent test material in the gastrointestinal tract. Other changes in animals found dead appeared to represent autolytic changes and/or antemortem stress. Changes in animals killed after 14 days were similar to those seen in control animals killed by carbon dioxide inhalation or were considered to represent physiological variation (Monsanto Company, unpublished report, 1986a).

A study using a substance Trilon A 92 (Na₃NTA) resulted in LD50 values of 1470 mg/kg bw for female and 2220 mg/kg bw for male rats: Doses of 1000, 1470, 2150 and 2610 mg/kg bw were administered to 4 groups of 5 male and 5 female rats per group by gavage using aqua dest. as vehicle. No deaths were observed after oral treatment with 1000 mg/kg. Two males and 3 females died after 1470 mg/kg within 2 days, 2 males and all females died after 2150 mg/kg and 3 males and all females died after 2610 mg/kg within 1 day. Surviving animals (observation period 14 days) exhibited dyspnea, apathy and poor general state at day 1. Animals treated with the higher doses showed abnormal position, staggering gait, twitching, opisthotonus, tonic convulsion and salivation in addition to dyspnea and apathy. At necropsy, animals that died within the study demonstrated hyperemic glandular stomach and mucosa

with multiple hemorrhagic erosions, in animals sacrificed after 14 days no abnormalities were detected (BASF AG, unpublished report, 1985).

Oral LD50 values for a 40% aqueous solution of nitrilotriacetic acid trisodium salt monohydrate were determined for rats, resulting in 3800 mg/kg bw for females and 5300 mg/kg bw for males (FIFRA/TSCA Methods of 1982): Single doses of 2500, 3500, 5000, 7100 and 10000 mg/kg of a 40% aqueous solution of Na₃NTA were administered by oral intubation to 5 groups of 5 male and 5 female rats each. No deaths occurred after treatment with 2500 mg/kg, 1 male and 4 females died within 24 hours after administration of 3500 mg/kg, 2 males and 4 females died within 4 hours after administration of 5000 mg/kg, all rats died after treatment with 7100 and 10000 mg/kg within 4 hours. Signs seen on the day of dosing in all or most groups included ataxia, tremors, hypopnea, hypothermia, hypoactivity, prostration, oral, nasal and ocular discharge wet rales, soft stool, unthrifty coats, fecal and urinary staining and decreased food consumption, except for isolated occurrences of unthrifty coat and the presence of unilateral ocular opacities in one animal. Surviving animals were free of significant abnormalities from day 2 through termination of the study on day 14. Postmortem examinations of animals found dead revealed a variety of changes, primarily in the lungs and gastrointestinal tract. Some animals had changes in the stomach and intestine which were suggestive of an irritant effect (red fluid in the stomach and intestine, discoloration of walls), and several had apparent test material in the gastrointestinal tract. Other changes in animals found dead appeared to represent autolytic changes. Changes in animals killed after 14 days were similar to those seen in control animals killed by carbon dioxide inhalation or were considered to represent physiological variation (Monsanto Company, unpublished report, 1986b).

A similar study with a 25% aqueous solution of the substance (Na₃NTA x H₂O) resulted in a LD50 value of 3715 mg/kg bw for rats: Doses of 2510, 3160, 3980 and 5010 mg/kg bw of the 25% aqueous substance solution were fed by stomach tube groups of 5 rats each (2 male and 3 female or 3 male and 2 female rats per group). One female each died in the 2510 mg/kg and in the 3160 mg/kg groups, all 3 females died in the 3980 mg/kg group (both males survived), 1/5 rats survived in the 5010 mg/kg group. The observation time after treatment was not stated but probably did not exceed more than one day. Toxic symptoms included weakness in 10-15 minutes, much discomfort and tremors in 29-50 minutes. At necropsy (no information on observation period), there was inflammation of the gastric mucosa and liver hyperemia seen macroscopically (Monsanto Company, unpublished report, 1968).

In a study using nitrilotriacetic acid trisodium salt (Na₃NTA) of unknown purity, an oral LD50 of 1680 mg/kg bw was found for a 20% aqueous substance solution: Groups of 5 male and 5 female rats per group were dosed with single doses of 998, 1400, 2060 and 2730 mg/kg of 20% aqueous solution. Following a 2-week observation period, survivors were necropsied and tissues were examined microscopically. Histological examination of tissues revealed no significant pathological findings (Nixon, 1971).

An oral LD50 of more than 5000 mg/kg bw revealed in a study with dogs receiving an 80% aqueous suspension of nitrilotriacetic acid trisodium salt (Na₃NTA, no data on purity): The aqueous suspension of the substance was administered by stomach tube to groups of 4 mongrel dogs at dosage levels of 1000, 2500 and 5000 mg/kg. Each dog was observed for emesis and toxic manifestations for at least 1 week. Results of emetic studies with beagle dogs demonstrated also potent and prompt emetic activity of the substance. No effect, other than emesis, was noted in the test animals (Nixon, 1971).

Inhalation

Within a report of the US Environmental Protection Agency on the toxicological hazards caused by NTA (EPA, 1980) several studies are reported that were performed in order to assess inhalation toxicity of trisodium nitrilotriacetate:

- 1) A pulmonary screening was performed with mice, which were exposed to NTA aerosols at 0.22, 1.09, 1.41 and 7.6 mg/l for 5 minutes. No death occurred. Slight sensory irritation was concluded for 0.22 mg/l, whilst moderate sensory irritation was concluded for 1.09 and 1.41 mg/l. Severe sensory irritation was concluded for 7.6 mg/l (no more details provided).
- 2) In a LC50 study, 10 male albino rats were exposed for 4 hours to:
 - (a) non-micronised NTA at levels of 3.3, 3.6 and 5.0 mg/l (no data on purity). 5mg/l was the maximal level attainable under dynamic exposure conditions using a cyclone dust generator.
 - (b) micronised NTA at 5.0 mg/l (no data on purity).

Animals were observed for 14 days. No mortalities were observed. Clinical signs included salivation, slow labored respiration, partially closed eyes and hypoactivity (no more details provided). After termination of exposure, all animals recovered and appeared healthy throughout the remaining observation period. Weight gain of exposed animals was normal and there were no pathological findings.

- 3) In a 4-day repeated exposure study, 10 male rats were exposed to concentrations of 0, 0.002, 0.02, 0.2 and 2 mg/l of micronized NTA for 6 hours/day on 4 consecutive days. Animals were necropsied after a 14-day observation period. Examinations on laboratory pathology and histopathology were not performed, data on strain, substance purity and measured concentrations were not reported. None of the NTA-exposed animals died, all high dose animals showed signs indicative for respiratory, nasal and eye irritation without any further detail on the onset and duration of the symptoms (see also under 4.1.2.6.1). In a 4-week repeated exposure study, monkeys, rats and guinea pigs were exposed to nominal concentrations of 0.01, 0.25 and 0.5 mg/L non-micronised NTA for 6 hours/day, 5 days/week for 4 weeks followed by a 2 week observation period. Measured concentrations were 0.013, 0.137, and 0.343 mg/l, respectively. At 0.342 mg/l, some rats (2/12) and guinea pigs (4/12) showed dyspnea during the first 2 weeks of exposure; the monkeys exhibited diarrhea but no respiratory irritation or general discomfort. No deaths occurred in any of these treatment groups (see also under 4.1.2.6.1).

Dermal

Data on the acute dermal toxicity of the substance are sparse, but sufficient to assess possible hazard: A minimum lethal dose of >10 000 mg/kg bw resulted for rabbits after skin contact with a 25% aqueous solution of nitrilotriacetic acid trisodium salt monohydrate ($\text{Na}_3\text{NTA} \times \text{H}_2\text{O}$): Doses of 1000, 1580, 2510, 3980, 6310 and 10000 mg/kg bw were applied to the closely clipped intact skin of one male or female rabbit per dose. The treated areas were covered with plastic strips and the animals held in wooden stocks for periods up to 24 hours, after which time they were assigned to individual cages (no other information on the test method used, especially no information on observation time after removal of the bandage). No nervousness or muscular incoordination developed. Activity and appetite were reduced for

2-3 days at the higher levels and there was mild weakness. No local symptoms were reported. Necropsy was not performed (Monsanto Company, unpublished report, 1968).

Human data:

Human data are not available.

Conclusion:

Human data on the acute toxicity of trisodium nitrilotriacetate are not available. An oral LD50 of approximately 750 mg/kg bw was determined for monkeys, of 1300-1470 mg/kg bw for female rats and of 1600-2220 mg/kg bw for male rats. The oral LD50 for dogs exceeds 5000 mg/kg bw. These LD50 values do not correspond to oral absorption values. In conclusion, the mechanism of systemic acute oral toxicity may be different in primates, rats and dogs. Symptoms reported in Rhesus monkeys indicate neurotoxicity, which was not reported in rats and dogs.

Data on inhalation toxicity demonstrate a low acute toxicity which does not require classification and labelling. In an inhalation study reported by the EPA (1980), a LC50 >5.0 mg/l was reported.

Acute dermal toxicity seems to be low as inferred from a test with rabbits resulting in a minimum lethal dose of more than 10 g/kg for rabbits treated with a 25% aqueous substance solution.

Based on the available data the substance is classified as „Xn, harmful“ and labelled with „R 22 - harmful if swallowed“. Acute dermal toxicity does not need labelling because the minimum lethal dose for rabbits was detected to exceed the limit dose of 2000 mg/kg for rabbits. At present, assessment of acute inhalation toxicity is not possible due to the lack of data.

4.1.2.3 Irritation and

4.1.2.4 Corrosivity

Animal data:

The currently available data on Draize tests with rabbits are not sufficient for proper assessment of skin and eye irritant/corrosive properties of pure undiluted trisodium nitrilotriacetate:

In a Draize skin irritation test with rabbits, unmoistened nitrilotriacetic acid trisodium salt monohydrate of unknown purity was used (finely ground dry powder): No skin changes were noted in three rabbits within an observation period of 7 days after occlusive application of the dry powder (no information on the amount used). The dry substance was applied to the clipped intact skin and removed after 24 hours (purity and amount of substance not specified). The application sites were covered with plastic strips to avoid contamination (Monsanto Company, unpublished report, 1968).

Mild skin irritation reversed within 5 days in a Draize test using a 25% aqueous solution of nitrilotriacetic acid trisodium salt monohydrate: The aqueous solution (no information on the amount used) was applied to the clipped intact skin of 3 albino rabbits and removed after 24 hours. The applications were covered with plastic strips to retard evaporation and avoid contamination. Findings were negative in the first hour. Overnight there was slight to well-defined erythema with one instance of slight edema. Redness nearly disappeared within 3 days. Mixed erythema/edema irritation mean scores for observations 24, 48 and 72 hours after patch removal were calculated (2.3/1.3/1.0). No local signs were observed after 5 days (Monsanto Company, unpublished report, 1968).

In a Buehler study with guinea pigs a 50% substance formulation in distilled water was used for topical induction and challenge. This concentration did not cause any sign of skin irritation (BASF, 1997a).

Mild eye irritation was noted in a Draize test according to OECD TG 405, when 0.1 mL of a 38% solution of nitrilotriacetic acid trisodium salt ("Trilon A fluid") was applied to the conjunctival sac of 3 albino rabbits. After 24 hours, average values for redness and chemosis of the conjunctiva were 2.0 and 0.7, respectively. After 48 hours, value for conjunctival reddening was 1.0 and no chemosis was present. Conjunctival reddening was abated after 8 days. There were no findings for cornea and iris (BASF, 1982).

Moderate eye irritation was detected in a Draize test using nitrilotriacetic acid trisodium salt monohydrate (no data on purity): One hundred mg of finely ground sample were placed in the conjunctival sac of each of 3 albino rabbits. Considerable discomfort was shown immediately upon application. Copious discharge, edema with partial eversion of the lids, moderate redness, and sufficient congestion to mildly obscure iris details was recorded after 1 hour. The eyes were rinsed with warm isotonic saline solution after 24 hours, when discharge and edema had already reduced. Edema disappeared within 5 days leaving mild redness and slight corneal dullness (no information on scores) which were observed also after 7 days when the study was terminated (Monsanto Company, unpublished report, 1968).

Respiratory irritation

Within a report of the US Environmental Protection Agency on the toxicological hazards caused by NTA (EPA, 1980) several studies are reported that were performed in order to assess inhalation toxicity of trisodium nitrilotriacetate (all available details presented in section 'acute inhalation toxicity').

- 4) A pulmonary screening was performed with mice, which were exposed to NTA aerosols at 0.22, 1.09, 1.41 and 7.6 mg/l for 5 minutes. Slight sensory irritation was concluded for 0.22 mg/l, whilst moderate sensory irritation was concluded for 1.09 and 1.41 mg/l. Severe sensory irritation was concluded for 7.6 mg/l.
- 5) In a LC50 study, 10 male albino rats were exposed for 4 hours to:
 - (c) non-micronised NTA at levels of 3.3, 3.6 and 5.0 mg/l (no data on purity). 5mg/l was the maximal level attainable under dynamic exposure conditions using a cyclone dust generator.
 - (d) micronised NTA at 5.0 mg/l (no data on purity).

Animals were observed for 14 days. Clinical signs included salivation, slow labored respiration, partially closed eyes and hypoactivity (no more details provided). After

termination of exposure, all animals recovered and appeared healthy throughout the remaining observation period.

In a 4-day repeated exposure study, 10 male rats were exposed to concentrations of 0, 0.002, 0.02, 0.2 and 2 mg/l of micronized NTA for 6 hours/day on 4 consecutive days. Animals were necropsied after a 14-day observation period. None of the NTA-exposed animals died, all high dose animals showed signs indicative for respiratory, nasal and eye irritation without any further detail on the onset and duration of the symptoms (see also under 4.1.2.6.1). In a 4-week repeated exposure study, monkeys, rats and guinea pigs were exposed to nominal concentrations of 0.01, 0.25 and 0.5 mg/L non-micronised NTA for 6 hours/day, 5 days/week for 4 weeks followed by a 2 week observation period. Measured concentrations were 0.013, 0.137, and 0.343 mg/l, respectively. At 0.342 mg/l, some rats (2/12) and guinea pigs (4/12) showed dyspnea during the first 2 weeks of exposure; the monkeys exhibited diarrhea but no respiratory irritation or general discomfort. No deaths occurred in any of these treatment groups (see also under 4.1.2.6.1).

Human data:

Human data are not available.

Conclusion:

Human data on local irritancy are not available. On the basis of the animal data currently available proper assessment of skin and eye irritant/corrosive properties of the substance is not possible. When evaluating the potential of NTA to cause skin and eye irritation, also the high pH of a 1% aqueous solution should be considered (pH 10.5 – 11.5, BASF Material Data Sheet 2004). There were no effects after a 24 hours application of the finely ground powder (amount not given). Effects of a 24 hours application of a 25% aqueous solution of NTA, which were reversible within 5 days, were not described (Monsanto, 1968). However, since added Draize scores did not exceed threshold levels defined for significant irritation, classification and labelling for skin irritation is not warranted. When assessing this result, also the prolonged application time of 24 hours (compared to a 4 hours exposure of a regular guideline test) should be considered. Moderate eye irritation was observed in a Draize test, which was not reversible within the study duration (Monsanto Company, unpublished report, 1968). Since the study was terminated on day 7, the reversibility of eye irritation on day 21 could not be assessed as required by standards. However, due to the nature of the effects, irreversibility is not to be expected. Another study indicated recovery from moderate irritation following application of a 38% Na₃NTA solution (BASF, 1982), but this study does not allow to conclude effects of the pure substance. Based on the limited data available, including the basic pH of a 1% aqueous solution, the current classification of R36 - Irritating to eyes - is confirmed.

Following acute and repeated inhalation of dust, Na₃NTA may exert some irritation of the respiratory tract as evidenced in mice and rats. Since significant reactions only occurred at highest concentrations in the range of the maximal technically achievable level, no respiratory irritation was reported after a 4-hours single exposure, no effects were reported for monkeys in a 4-week study and there is no evidence for respiratory tract irritation in humans, these

findings do not warrant classification as “Irritating to respiratory system“ and labelling with R 37.

4.1.2.5 Sensitisation

Skin sensitisation

Animal data:

Trilon A 92 (Na₃NTA, purity 92.4%, white powder) was tested in a Buehler test using guinea pigs (20 animals induced with NTA + 10 not-induced animals in control group). For induction and challenge treatments a 50% substance formulation in distilled water (0.5 ml) was used. After three induction treatments at day 0, 7 and 14 no skin irritation was noted. After challenge none of the treated or control animals showed skin reactions (BASF, 1997a). A positive control study (reliability check) was not performed within this study. The most recent control study using 85% α -hexylcinnamal aldehyde in the same year showed valid results. However, this study shows a significant shortcoming: a 50% formulation of NTA was used for the dermal inductions. However, the prepared formulation did not cause any sign of skin irritation, which is a prerequisite when applying this method. In consequence, the negative result of this study cannot be used for risk assessment.

Human data:

Closed patch test studies were carried out with 66 volunteers using a 1% aqueous solution of a liquid detergent containing 20% Na₃NTA. The test material was applied in 0.5 ml quantities to $\frac{3}{4}$ x 1 Webril padding, placed on the upper arm of human subjects, and occluded with Elastoplast. A total of nine serial applications three times a week of three consecutive weeks, followed by a challenge two weeks after the last insult, constituted the testing procedure. Patches were removed by the subjects 24 hr after application; skin reactions were graded by trained observers after 48 and 96 hr. Challenge patches with the same concentration of the test material were placed on both the original site of insult and on an alternate site of the opposite arm, to aid the differentiation of skin fatigue and sensitization. No evidence of sensitization was noted in these volunteers (no more details on results provided; Nixon, 1971)

Respiratory sensitisation

no data available

Conclusion:

A Buehler guinea pig study, conducted in 1997, demonstrates a negative result. However, the test procedure was not valid, since a non-irritating substance formulation was used for dermal inductions. Therefore, the negative result obtained with the Buehler test cannot be used for risk assessment purposes.

The negative data on patch tests with volunteers, published in 1971, is not considered to be of sufficient evidence that the substance may not cause contact allergy to a sensitive subpopulation, since this study is based on a rather limited number of participants. Thus, the negative data obtained in 1971 above cannot be considered as evidence that the substance may not cause sensitization by skin contact. On the other hand, there exist no human case reports which point to a potential of NTA to cause skin sensitisation.

However, balancing the comparably low effort and demand of test animals, it may be considered to conduct a LLNA in order to reduce uncertainty of existing data und to fulfil Annex VII requirements.

Additional QSAR considerations and “weight of evidence approach”

In order to close the existing data gap for skin sensitisation, a QSAR analysis was provided (for details refer to Appendix 1).

Weight of Evidence approach for skin sensitisation

The outcome of the Buehler test was negative, which indicates a lack of a strong skin sensitisation potential of NTA. However, to finalise risk assessment, additional data have to be considered. The bioavailability of NTA for sensitisation is limited because it is a salt and ionised chemicals have limited dermal penetrations (expert judgment). On the other hand the molecular weight of 257.1 g/mol does not exclude dermal penetration. A recent skin penetration study has demonstrated a low dermal penetration rate in vitro (BASF, 2007b). According to a DerekfW prediction, the chemical reactivity and so the binding to proteins is limited. Generally speaking, the outcome of DerekfW for the absence of sensitisation has limited weight, because negative findings usually have to be considered out of the applicability domain. However, viewing the alerts present in DerekfW this type of chemicals may be considered negative. Protein binding is considered limited, because the ester-salt is not activated and the amine is tertiary which may be sterically hindered (expert judgment). The chemical is within the TOPKAT domain and provides an analogue which is negative. Though the TOPKAT models may have some uncertainty because of lack of mechanistic reasoning, the analogue approach can add to the overall impression that the chemical is not a sensitizer. Additionally, 3 structurally closely related substances have been identified, which consistently gave negative results for skin sensitisation in guideline tests (EU new substances notifications, confidential data). EDTA, which can also be considered to have structural characteristics in common with NTA, also gave negative results for skin sensitisation (Guinea pig maximisation test). Overall, it can be concluded that there is no evidence to conclude a skin sensitising potential for NTA and additional testing may be waived.

Overall conclusion: no classification and labelling to be performed.

4.1.2.6 Repeated dose toxicity

Studies with administration of NTA in its forms as Na₂NTA, Na₃NTA.H₂O, and H₃NTA were considered as relevant information because these compounds dissociate into the nitrilotriacetate anions and the respective cations under physiological conditions. Data from

studies with the following soluble, but strongly associated complexes of CaNaNTA, MgNaNTA, AlNTA, ZnNaNTA or FeNTA were discarded from this section of the report.

The study order follows the administration route (inhalation<oral (via gavage<diet<drinking water)<dermal<intraperitoneal), the duration of the study (short term<long term) and the species (rat<mouse<dog<rabbit).

4.1.2.6.1 Animal studies

Animal studies with inhalation exposure:

RAT - Subacute toxicity

- A communication to the EPA (1980) containing summarised data only reported that groups of 10 male albino rats were exposed to concentrations of 0, 0.002, 0.02, 0.2 and 2 mg/l of micronized NTA for 6 hours/day on 4 consecutive days. Animals were necropsied after a 14-day observation period. Examinations on laboratory pathology and histopathology were not performed, data on strain, substance purity and measured concentrations were not reported. None of the NTA-exposed animals died, all high dose animals showed signs indicative for respiratory, nasal and eye irritation without any further detail on the onset and duration of the symptoms. On day 14 of recovery all treated animals appeared normal with respect to the clinical and necropsy findings. Based on the clinical symptoms, the NOAEC for local effects observed after a 4-day inhalation exposure on rats was 0.2 mg/l (0.0002 mg/m³) of NTA.

OTHER SPECIES - Subacute toxicity

- Another study of this EPA report (1980), a 4-week exposure of rats (12/dose level), guinea pigs (12/dose level) and monkeys (4/dose level) on 6 h/d, 5 days/week, to measured concentrations of 0, 0.01, 0.21 and 0.34 mg/l NTA (non-micronized) is cited to result in dyspnoe during the first 2 weeks of exposure in 2 rats and 4 guinea pigs at the high dose level. Monkeys at this dose level exhibited diarrhea. Incidental mortalities and necropsy findings were not attributed to the NTA exposure (no detailed data). Growth curves were comparable to the controls during the treatment period and the 2-week observation period. No abnormalities were reported for hematology examinations (no data on parameters determined), whereas the rat serum values of ASAT activities, albumin, globulin, total protein and gamma globulin were increased at the end of treatment. With the exception of gamma globulin all values had returned to control range by day 42. In monkeys, no adverse effect on the pulmonary function was estimated on total respiratory system flow resistance, N₂ washout and rate of CO diffusion. Data were absent for the strains and sexes tested, substance purity, parameters of laboratory pathology, organ weights, and microscopic examinations. Under consideration of the incompleteness of the study comparing to standard test protocols, the NOAEC for local effects was 0.21 mg/l for rats and guinea pigs and 0.34 mg/l for monkeys. The NOAEC for systemic effects was 0.21 mg/l (0.00021 mg/m³) for the monkey and rat, and 0.34 mg/l (0.00034 mg/m³) for the guinea pig.

Animal studies with oral administration:

Gavage studies

RAT - Subacute toxicity

- One of the most recent study on NTA was an oral 3-week study on Wistar rats (BASF, 1997b). H₃NTA (purity 98-99%) was administered daily to groups of 5 male and 5 female rats by gavage at dose levels of 150, 500, and 1500 mg/kg bw/d. A control group received the vehicle (0.5% aqueous carboxymethyl cellulose solution) only. The test protocol was not equivalent to the standard protocol for the oral 28-day study in OECD 407, since the study duration was only 3 weeks, the blood chemistry examinations were limited to urea and creatinine, hematology parameters were not examined, and histomorphologic examination was conducted on the kidney only. In addition, one male and one female of each group were perfused with formaldehyde solution and the kidneys were examined by electron microscopy with the purpose to characterise the tubular degeneration.

There was no unscheduled death during the study. Lower feed consumption was observed in high dose males from day 7 to the end of study, and in high dose females only at day 7. The consumption of feed was dose-related reduced in males of the mid and low doses; however, they were reported to be within the normal range for these animals at that age. A dose-related lower body weight and lower body weight gain were observed in treated males gaining significance at the mid and high dose levels. In the mid and high dose females, a transient reduction of the body weight and body weight gain was observed on day 7 of the study. Diarrhea, anogenital region smeared with urine, crust formation at the nose and piloerection were observed in high dose males and females. These groups showed a tendency to higher urea concentrations, but no clear effect on creatinine levels was seen. Urinalysis revealed non-significantly lower pH values in males in a dose-related manner and in high dose females. Increased blood and erythrocytes were detected in the urine specimens of the high dose males, whereas increased renal tubular and transitional epithelial cells and granular casts were observed in the urine of the high dose females. The mean values of absolute kidney weights were significantly higher in high dose females compared to the control group. Significantly elevated relative kidney weights were found in high dose males and females of the mid and high dose groups. Increased kidney weights relative to body weight were also seen in male rats of the mid dose group, but the increase did not gain significance. Enlarged and discoloured kidneys were observed in each one male and female of the mid and high dose groups. A white focus was noted in one kidney of a high dose male, this lesion was confirmed to be an area of severe tubular hyperplasia.

Light microscopy of the kidneys revealed tubular vacuolation, often multifocal and bilateral, in most males and females of the mid and high dose groups. Males were more affected than females, and mean severity of this lesion increased with dosage. A diffuse degeneration of the pars recta of the proximal tubule was present in three high dose males and one mid dose male. A focal degeneration of the pars recta was observed in one high dose female. The incidence of low graded basophilic (regenerating) tubules was similar between treatment and control groups, whereas the incidence of this lesion with higher severity grades increased with dosage. In addition to the focus in one high dose male confirmed to be an area of severe tubular hyperplasia, another hyperplastic lesion of the tubules was noted in a second high

dose male. This lesion consisted of hyperplastic basophilic and clear cell tubules. Further findings as mononuclear cell infiltration, interstitial fibrosis, proteinaceous casts, focal glomerulopathy and calcification at the cortico-medullary junction were similarly distributed between the treatment and control groups.

Ultrastructural examinations did not show alterations of the pars recta of the proximal tubule in animals of the low and mid dose groups. A prominent vacuolation of tubular cytoplasm of a high dose male was characterised as a vacuolation and vesiculation of the rough endoplasmatic reticulum due to increased water influx. Roundish, sometimes irregular shaped vacuoles were observed in the cells of the third segment of the proximal tubule. Electron-lucent vacuoles of different sizes were regularly distributed in the cytoplasm and were limited by a singular membrane. Electron-dense structures with a diameter of 10 nm identical to the morphology of ribosomes were free or in close contact to the vacuolar membranes. Vacuolated cells appeared to have fewer cell organelles such as mitochondria and lysosomes, the content of strands of the rough endoplasmatic reticulum was markedly reduced. The observed vacuolar degeneration was regarded a consequence of acute cellular swelling which finally results in cell death. No adverse effect was observed at the dose of 150 mg/kg H₃NTA (NOAEL).

- To focus on the sequential steps in the development of renal damage, NTA as the Na₃NTA.H₂O salt in deionised water was administered by gavage to male Sprague-Dawley rats at levels of 0, 0.73 or 7.3 mmol/kg bw/d (appr. 0, 187.6 or 1876 mg/kg bw/d) for periods up to 30 days (Merski, 1982). Two animals from each of the groups were killed 24 hours after dosing on day 9, 13, 16, 20, 23, 27 or 30. Cytoplasmic vacuolation and hyperplasia of the proximal convoluted tubules were the most prominent alterations being observed from day 9 on. The number of affected cross sections showing cytoplasmic vacuolation was much greater in the kidneys of rats given 7.3 mmol/kg Na₃NTA.H₂O than in those given the lower dose. A higher incidence of basophilic tubules and of basophilic simple tubular hyperplasia was registered in both NTA-treated groups compared to the control group. Simple tubular hyperplasia associated with vacuolation increased in a dose-related manner and was only observed in NTA-treated rats, but not in control animals. Except one case in the low dose group, the development of tubular nodular hyperplasia was observed only in high dose rats. In addition vacuolation of epithelial cells were found in over 90% of the nodular hyperplastic lesions, the remaining were basophilic. Beginning at day 13, changes in the renal pelvic transitional epithelium were observed in high dose animals. The development of focal haemorrhage, necrosis, erosion, and hyperplasia of the epithelium of the renal pelvis were the most prominent lesions. The LOAEL for the adverse effects on the kidney was 0.73 mmol/kg bw/d (appr. 187 mg/kg bw/d).

Feeding studies:

Comment on the dose calculation in rat feeding studies: If not otherwise mentioned, calculated intake of the test substance is based on an average food intake of 7% of the body weight per day. If no data on the purity of the test substance is reported, 100% purity is assumed.

RAT - Subacute toxicity

- In a recent report, the kidney toxicity of $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ after repeated oral administration were compared to that of FeNTA after repeated intraperitoneal injections (data not reported here) (BASF, 1998). $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ (92.4%) was administered to 14-week old male Wistar rats at dietary concentrations of 0, 150 and 20000 ppm (appr. 9 and 926 mg/kg bw/d) for 4 weeks. Subgroups of 5 animals received 150 ppm (2 groups), 20000 ppm (4 groups) or served as controls (4 groups). Animals were examined for clinical observations, food consumption, body weight (change), serum transferrin, iron, total iron-binding capacity, urinalysis on day 3 before treatment and on days 2, 4, 8, and 29 of treatment, hematology on day 30, histopathology of several organs and ultrastructural pathology of the kidneys, determinations of 8-OH-2-deoxyguanosine and of lipid peroxidation in kidneys, as well as determination of DNA-synthesis in the kidneys. The last three examinations will be reported in 4.1.2.7. In addition to standard urinalysis including the semi quantitative strip method, an extended quantitative urinalysis was performed on creatinine concentrations and enzyme activities of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and alanine aminopeptidase (AAP) and N-acetyl- β -glucosaminidase (NAG) were measured. Urinary calcium, iron and zinc were also measured. Due to the missing hematology examinations, the incomplete clinical biochemistry, and the incomplete list of organs examined for histopathology, this study did not fulfil the requirements of current standard test protocols of the OECD 407/EEC B.7 method.

Groups of 5 males of the control and both treatment groups were sacrificed by perfusion fixation and specimens of the liver, spleen, kidneys, pancreas, urinary bladder, and ureter were embedded in paraplast. H&E staining were prepared of sections of the kidneys, spleen, liver and pancreas, other target sites such as the ureters and the urinary bladder were not examined microscopically. Sections of the kidney, spleen and liver were stained with Perl's iron staining of Fe^{3+} and Fe^{2+} . The Turnbull iron staining of Fe^{2+} was also performed on kidney and spleen section. In addition, plastic embedded semithin sections of five organ samples of each kidney representing the areas of renal cortex, the outer and inner medulla were examined. Representative samples of different kidney compartments of all test animals were examined electron microscopically.

At 20000 ppm Na_3NTA group one animal died on day 28, all animals had red-brown discoloured urine from day 9 to the end of the study, some of the animals showed piloerection from day 21 on. At this dose level, food consumption was decreased up to 48% during the entire study and water consumption increased up to 102% of the controls. The body weights were significantly lower (-26%) than in the control groups. Clinical chemistry examinations did not reveal changes of the levels of iron concentrations, transferrin levels and total iron binding capacity. Quantitative measurements in the urine revealed increased activities of LDH on day 2, 4 and 8 of the study. At day 8, γ -GGT and creatinine concentrations were reduced. Increased concentrations of zinc were excreted with the urine at the end of the study, but calcium and iron excretion remained unaffected. Standard urinalysis revealed macrohematuria on day 8, increased blood and in the sediment elevated number of erythrocytes on day 4, 8, and 29. The mean specific gravity was reduced and the mean urine volume was increased (none significantly) on day 8 and 29. The urine specimens were discoloured from dark yellow to light brown and appeared cloudy on days 2, 4 and 8. Decreased amounts of urinary crystals were found on day 2, 4 and 8 and increased numbers of transitional epithelial were found on day 4 and 8 of the treatment. All animals of this dose group showed enlarged kidneys and dilation of the renal pelvis. The ureters were dilated in

one animal. Microscopic lesions were seen in the kidney of all high dose animals consisting of tubular hyperplasia. These hyperplasias were characterised by tubules with large, vacuolized cells. Other changes in most or all animals of this group were basophilia, vacuolation (without hyperplasia), dilation, and calcification of tubules, interstitial nephritis, inflammation, necrosis, fibrosis and urothelial hyperplasia of the renal papilla and pelvic dilation.

The severity of hyperplastic lesions was associated with the occurrence of pelvic dilation and loss/necrosis of the renal papilla. Iron particles of interstitial macrophages positive with Perl's iron staining (Fe^{2+} and Fe^{3+}) were similarly distributed between the treated and control groups. No positive reaction was found with Turnbull's stain (Fe^{2+}) indicating that the observed particles were likely to represent hemosiderin.

Ultra structurally, the histomorphological vacuolation of tubular epithelial cells were confirmed to consist of different stages of vesiculation and dilation of the rough endoplasmic endothelium, occasionally accompanied by cytoplasmic blebbing into the tubular lumen, and of ballooning degeneration of mitochondria. These changes were found in samples of the cortex and outer medulla, but not in the inner medullar regions. They are characteristic for different stages of cell swelling and vacuolar degeneration up to lysis of cells.

No renal lesions except a single animal with tubular calcification and two animals with mononuclear cell infiltration were observed in the low dose group. No lesions related to the Na_3NTA treatment were observed in the liver, pancreas, and spleen.

The observed lesions of other subgroups were in accordance with the described and therefore not separately reported. The NOAEL of this study was 150 ppm $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ (9 mg/kg bw/d).

- Weanling female rats (Sprague Dawley, 60 g) were fed with diet containing 0, 0.5, 0.75, 1.5 and 2% of $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ (0, 350, 525, 1050, and 1400 mg/kg bw/d) or 0, 0.5, 0.75 and 1.5% H_3NTA (0, 350, 525, and 1050 mg/kg bw/d) for 4 weeks (Anderson & Kanerva, 1978). The effects on growth, urine pH and urine volume were measured. The urines from the animals consuming both forms of 0.75% NTA contain insoluble material and haematuria was observed in the urines of rats at 1.5% $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$. X-ray analyses showed that the insoluble material contained crystalline CaNaNTA (not quantified). All doses of $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ reduced growth and increased urine pH above the controls. Urine volume was reduced at 0.5% but higher doses increased the volume dose-dependently. - Lower urine volume was measured at 0.5% H_3NTA and higher, but decrease did not show dose-relationship. Body weight reduction and decreased urine pH-values were seen at 0.75% and above. Related to the limited parameters under investigation, no NOAEL was estimated for both NTA forms. 0.5% (350 mg/kg bw/d) of $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ or H_3NTA was considered as LOAEL.

Additional investigations demonstrated that the effect of NTA dose on urinary Ca measured in male and female Sprague-Dawley rats output was increased dose-dependently after 3 weeks feeding of 0.6 mol/100 g bw/day (1540 mg/kg bw(d) and above of $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ or H_3NTA (Anderson and Kanerva, 1978).

Adult male rats weighing about 250 g and fed diets containing both forms of NTA for 2 weeks were measured for urinary Ca and NTA output (Anderson and Kanerva, 1978). When the urinary NTA levels were 0.1 mol/100 g bw/d or less, urinary Ca output did not differ from controls. When urinary NTA exceeded 0.1 mol/100 g bw/d, urinary Ca output increased

linearly until urinary NTA was 0.17 mol/100 g bw/d. Higher urinary NTA levels further increased urinary Ca levels only slightly.

- NTA effects on the growth, kidney weight and urine were examined in another 4-week study on 5 male and 5 female Sprague Dawley rats and 10 male and 10 female Fischer-344 rats which received a diet containing 1.5% H₃NTA or 2% Na₃NTA.H₂O (appr. 0, 1050 mg/kg H₃NTA or 1400 mg/kg Na₃NTA.H₂O) (Anderson & Kanerva, 1979).

Urine pH, volume, and the presence of precipitates and blood were determined on 24 h samples collected on days 4, 11, 18 and 25. The urines voided on day 25 by individual Sprague Dawley rats and pooled samples from F-344 rats were assayed for NTA content. The urine samples on day 18 were assayed for their Ca content. On day 29 all animals were necropsied and kidneys were weighed. Both forms of NTA reduced weight gain in all groups and food efficiency in males of both strains fed with Na₃NTA.H₂O and F344 males and females fed with H₃NTA. Growth reduction was greater in the F344 animals than in Sprague Dawley rats although the latter were consuming at least 1.6 times as much NTA as the F344 rats. Both strains and sexes developed crystalluria and haematuria. Kidney: body weight ratios were increased in the Na₃NTA.H₂O treated animals, but increase was only minimal in H₃NTA groups. Elevated urinary volume and increased urine pH was observed in Na₃NTA.H₂O fed rats. In contrast H₃NTA reduced urine volumes in male rats, had no consistent effect on females and reduced the urinary pH in all cases. The urinary concentrations of Ca varied considerably but all groups had elevated Ca levels. Renal excretions of NTA showed two-fold variations, values were in the 30-40% range of the ingested dose for all groups. At necropsy, only one F344 female of the Na₃NTA.H₂O groups developed hydronephrosis, whereas Sprague Dawley rats presented hydronephrosis in 40% of females and 100% of males of the Na₃NTA.H₂O groups and in 60% of males receiving H₃NTA.

Kidney histomorphology of the five male Sprague-Dawley rats associated to the feeding of 2% Na₃NTA.H₂O for four weeks was examined (Alden et al., 1981). A group of five male rats received control diet only. Treatment-related effects consisted of mild to severe cytoplasmic vacuolation of convoluted tubular epithelium and in more severely affected rats, additional evidence of convoluted tubular injury was granular degeneration and necrosis. The preferred site of vacuolation was the proximal convolutions of the nephrons. Hyperplastic tubules of the simple type lined with a vacuolated epithelium also occurred, and in three of five rats, the hyperplastic response was exaggerated resulting in the formation of hyperplastic tubular nodules. Other cortical alterations were observed spontaneously in control animals and in treated rats. The predominant alteration in the renal pelvis was erosion of the transitional epithelium in every treated rat progressing to ulceration in two rats. Sequelae to the epithelial erosion and ulceration were epithelial and subepithelial neutrophil infiltration, hemorrhage, fibrosis, and epithelial hyperplasia. Inflammatory and proliferative disease of the renal medulla was secondary to pelvic epithelial inflammation. In moderate-severe hydronephrotic kidneys, dilatation of collection ducts occurred in the medulla as well as in the cortex. In contrast to nephrocalcinosis that spontaneously occurs at the corticomedullary junction, mineralization in NTA receiving rats was observed as intracellular granules in convoluted tubular epithelium, and diffusely in subepithelial location in the renal papillae. Hydronephrosis was absent in control rats, and no other kidney lesions were reported. The only dose tested in this study with emphasis on renal effects represented the LOAEL (2% Na₃NTA.H₂O (1400 mg/kg bw/d) and 1.5% H₃NTA (1050 mg/kg bw/d)).

- Pelvic dilatation was visualised in pyelograms after intravenous application of contrast medium to 5 male Sprague Dawley rats fed with 2% Na₃NTA.H₂O (appr. 1400 mg/kg bw/d) and in 5 male F-344 rats fed with 3.5% Na₃NTA.H₂O (appr. 2450 mg/kg bw/d) for 28 days (Kanerva et al., 1984). These dietary concentrations were chosen to achieve nearly equivalent rates of ingestion of Na₃NTA.H₂O because of the higher food consumptions of Sprague-Dawley rats in the study of Anderson & Kanerva (1979). Both diets reduced body weight gains and elevated kidney: body weights in each of the strains (F-344 more affected than Sprague Dawley). In both strains ingested Na₃NTA.H₂O produced a marked increased size of the renal pelvis (hydronephrosis) and dilation of the proximal ureters. Microscopically, Na₃NTA.H₂O induced epithelial thickening and surface epithelial erosion was observed in the ureters. No histopathology was reported on other tissues. Restricted on the limited parameters of this study, a NOAEL was not estimated, the LOAEL is 2% Na₃NTA.H₂O (appr. 1400 mg/kg bw/d).
- In order to determine the effects of feeding Na₃NTA on the mineral excretion, Sprague-Dawley rats were fed with diet containing 2% Na₃NTA (appr. 1400 mg/kg bw/d) (4 males & 6 females) or normal diet (10 females & 6 males) for a period of 30 days (Michael & Wakim, 1973). Food consumption and body weight gain were recorded weekly. At the end of the 30-day feeding period, urine and feces were collected separately for 3 days. The parameters of urinalysis were pH, specific gravity, the contents of Zn, Ca, Fe, P, Na and K in ashed urine and the content of Zn, Ca, Fe, P and Na in ashed feces were determined. Mineral contents of excreta, tissues, serum and feed were determined with atomic absorption spectroscopy.

Animals receiving 2% Na₃NTA had lower growth rates and feed efficiencies than the control animals. Na₃NTA caused a significant increase in urinary pH (mean values of 8.22 and 8.42 in treated males resp. females vs. control values of 6.36 and 6.88), but changes in urinary excretion rate and specific gravity were not statistically significant. There were significant increases in urinary excretion of Ca, Zn and Na, but not in others minerals examined. The fecal excretion of Ca and P was decreased for both sexes, and the decrease in fecal excretion of Zn was significant only in females. Zn excretion rates of treated males were lower than in controls, but did not gain significance. No significant changes were seen in fecal (and urinary) Fe excretion. The Zn concentration in the sera of females that received Na₃NTA was significantly above the control. No significant differences were seen between the treated males and controls for Zn and for the Ca, Fe or Na in sera of both sexes. Related to the effects on mineral homeostasis, a NOAEL was not established, the LOAEL is 2% Na₃NTA.

RAT - Subchronic studies:

- The reversibility of NTA-associated nephrotoxicity was investigated by comparing renal tissues from male Sprague-Dawley rats (6-8 males/group) fed nephrotoxic levels of NTA for 7 weeks with those from rats allowed 5 wk of recovery after the 7-week exposure (Myers et al., 1982). Animals obtained as weanlings received 2% Na₃NTA.H₂O in the diet (73 µmol/g diet, appr. 1309 mg/kg bw/d), or 1.5% H₃NTA (79 µmol/g diet, appr. 1050 mg/kg bw/d) or control diet. Throughout the study weekly weight gains and feed consumption were measured. At necropsy at the end of exposure or recovery periods the left kidney were fixed by retrograde vascular perfusion and processed for histopathologic examination. In comparison to the control group, the two forms of NTA resulted in comparable decreases in growth rate during

the exposure phase of the study, these animals gained more weight during the recovery phase. Both forms induced vacuolation and hyperplasia often composed of vacuolated cells in the epithelium of the proximal convoluted tubules. These effects were noted in all of the exposed animals although the extent of damage varied. Tubular vacuolation was evident in each animal of the NTA groups, but was absent in the control groups and in the recovery groups indicating complete recovery. The total incidence of (simple) basophilic cell hyperplasia was greater in the NTA groups than in control groups after treatment and recovery period. Tubules that had a diameter more than twice of that of a normal proximal convoluted tubule were classified as tubular hyperplastic nodules of the vacuolated cell type. Nodular basophilic cell hyperplasia was only seen in treated groups and was absent in the control and recovery groups. Pelvic epithelial and subepithelial inflammation and fibrosis were observed in the groups given either form of NTA for 49 days. Mild to severe hyperplasia of the transitional epithelium (TE) in the renal pelvis occurred in 4/7 animals given diets containing Na₃NTA.H₂O and moderate TE hyperplasia was observed in 1/8 rats given H₃NTA in the diet. Animals with TE hyperplasia also exhibited pelvic dilation and TE erosion, ulceration and haemorrhage. TE dysplasia, intracytoplasmic globules, and mitotic figures were also noted. Tissues from recovery animals showed no abnormal cellular morphology in the pelvic urothelium in the presence of persisting hydronephrosis. In conclusion, NTA treatment was related to vacuolation, simple and nodular hyperplasia of the vacuolated cell type and the basophilic cell type in the proximal convoluted tubules, and transitional cell erosion, ulceration, hyperplasia and dysplasia associated with hydronephrosis in the renal pelvis. Nodular hyperplasias and urothelium lesions were shown to be reversible within this model, whereas simple basophilic hyperplasia of tubules and pelvic hydronephrosis persisted.

Vacuolation and basophilia of tubular cells with or without increased cell size is known to be a response to tubular injury. Lesions observed at the pelvis area are also characteristic for degenerative and regenerative response of urothelial cells. The authors discussed the higher incidence of basophilic cell hyperplasia being nonspecific and related to spontaneous age-related nephrosis. They were considered to be exacerbated by the ingestion of high doses of NTA. Although no data were reported for the acclimatisation period, it is assumed that rats were about 8±2 weeks at study begin. At the end of study, rats were about 15±2 weeks old. Therefore the role of spontaneous chronic progressive nephropathy of the old rat could not be clarified at this relatively young age and by the low number of animals of males only examined.- With respect to the renal damage, no NOAEL was established, the LOAEL was 2% Na₃NTA.H₂O (appr. 1309 mg/kg bw/d) respectively 1.5% H₃NTA (appr. 1050 mg/kg bw/d).

- In another shortly summarised report, a diet with 0, 2000, and 20000 ppm Na₃NTA (reported to be equivalent to appr. 0, 200, and 2000 mg/kg bw/d) was given to groups of 10 male and female rats (no data on strain) for 90 days (Nixon, 1971). A similar 90-day feeding study consisting of Sprague Dawley rats (40 males and 40 females/group) were fed meal containing 0, 7500, and 10000 ppm Na₃NTA (reported to be equivalent to appr. 0, 750, and 1000 mg/kg bw/d).

Body weights, feed consumption, 5 males and 5 females of each group of the first study and 10 males and 10 females of the second study were randomly selected for necropsy. Haematologic examination and histologic examination of 15 organs/tissues were performed in both studies.

Rats receiving a diet with 20000 ppm Na₃NTA showed reduced body weight gain, increased relative weights of the liver and the kidneys. In this group, abnormal kidneys which were enlarged and showed a rough uneven surface and microscopically, hydronephrosis was observed in 9/10 male and 3/9 female rats. The RBC counts (both sexes) and the hemoglobin content (males) were significantly reduced. Nothing abnormal was reported for rats fed with a diet containing 2000 ppm Na₃NTA.

In the second study (Nixon, 1971), significantly increased kidney/body weight ratios were seen in all test animals. The relative liver weights were also dose-related increased (without gaining significance) in treated males. Histologic examination showed mild hydropic degeneration of the tubular cells (in 4/10 male rats), and tubular atrophy and dilatation in two other males of the 7500 ppm group. More extensive kidney changes of the same nature were seen in animals receiving 10000 ppm Na₃NTA (no exact data on incidences reported). Blood levels and other organs did not show significant differences from controls. Changes of kidney and liver weights were thought to relate to toxicity of NTA rather than being a primary effect related to lower body weight gain, because no effect was seen on the weight of other organs, and kidneys and liver were affected at the mid dosage without any change of body weight gain in these animals.

Limited to the parameters tested, the NOAEL of this 90-day feeding studies is 2000 ppm Na₃NTA (appr. 200 mg/kg bw/d).

RAT - Chronic studies:

- Male and females Sprague-Dawley rats have been fed diets containing either 0, 0.03, 0.15 or 0.5% Na₃NTA (appr. 0, 19, 97, 322 mg/kg bw/d, purity of Na₃NTA 92.2%) or 0.5% of the calcium chelate of NTA (CaNaNTA, data not cited here) for up to 2 years (Nixon et al., 1972). At 6, 12, 19 and 24 months, 5 animals of either sex were randomly selected for metabolic and histological studies of kidney lesions. Additional tissues taken for histologic examination after 19 and 24 months were spleen, liver, lung, heart, stomach, esophagus, small intestine, adrenal, trachea, urinary bladder, gonads, thyroid and bone (however no data were reported on the absence of evidence of abnormalities in these organs). The femurs were analysed for Zn and NTA content and other parameters (dry and ash weight, percent ash, fat phosphorus, copper, magnesium, calcium).
- The feeding did not affect food consumption, feed efficiency, or growth of any test group during the first 8 weeks of the study (no further record thereafter). There was a dose-related trend in survival rates in dosed Na₃NTA-males, the survival rate was significantly lower in the high dose group. Liver body weight ratios were significantly higher than control values at 12 months for females receiving diets containing 0.5% Na₃NTA. A statistically significant increase in urinary zinc was intermittently noted in some rat groups receiving diets containing 0.15% and 0.5% Na₃NTA. In comparison to the control values, there was also a significant increase in the zinc level of bone in all test groups at nearly all check points during the experiment. The NTA deposition in the bone increased related to the dose, remaining constant during treatment and without any difference between sexes. The breaking strength of the bones from treated rats was not significantly altered from that of the control animals. No differences were noted microscopically in bones from test and control animals. In mid and high dose animals, lesions started at 6 months in 1 or 2 animals/group consisting of hydropic

degeneration of the tubule and minor tubule dilatation. At 12 months they were still considered to be mild, although they were more pronounced and were apparently test related since 4/10 animals were affected by the 0.5% Na₃NTA and 1/10 by the 0.15% Na₃NTA dietary level. In addition to early changes tubular dilatation with low basophilic epithelium, hemosiderin in the tubular epithelium and proteinaceous casts in the collecting tubules were also apparent. The rats fed 0.03% Na₃NTA did not exhibit these lesions and showed no differences from the control animals. At 24 months, moderate to severe chronic interstitial nephritis and nephrosis were observed with dose-related increase of incidence and severity in the mid and high dose groups of Na₃NTA fed males and females (total incidence 42% and 55%). Chronic renal lesions observed in 32% of the 0.03% Na₃NTA groups did not show significant differences from that of control animals (28%). Tumors of various types were reported to be similar for all groups and were considered not to be related to the treatment groups (details of incidences absent except for mammary gland tumors).

The NOAEL for renal toxicity was 0.03% Na₃NTA in diet (19 mg/kg bw/d) in this 2-year study.

MOUSE - Subchronic studies

Some laboratory findings were reported in a initiation-promotion study on male C3H/He mice (Matsuki et al., 1992, see also 4.1.2.8). Groups of 9-10 mice received Na₃NTA.H₂O or H₃NTA at concentrations of 1% with the diet at an age of 14 weeks for a period of 12 weeks. At week 20, the mean values of BUN, LDH, ASAT and ALAT activities were significantly elevated compared to the levels of 10 untreated controls. Data are too fragmentary in order to derive a N/LOEAL level.

DOG- Subchronic studies

- In a subchronic 90-day study, groups of 4 male and 4 female beagle dogs were fed with diet containing 0, 0.03, 0.14 and 0.5% of Na₃NTA (equivalent to medium uptake of 11, 42.5, and 130 mg/kg bw/d (Budny et al., 1973). No unscheduled death occurred, no test-related behavioral, physical or ophthalmologic changes were noted. Food consumption and body weight gains were comparable among control and treated dogs. No changes of hematologic or biochemistry parameters or in the serum content of Na, K, Ca, Mg, Fe, Zn, Co, and inorganic phosphorus considered to be test-related were observed. A comparison of the urinary cation excretion showed that the mid- and high-dose dogs had a significantly higher mean zinc excretion (with a high interindividual variability) than that in the control group (without dose-relationship in females). There was no significant difference in the excretion of Ca, Mg, Fe, Na, and K. No significant difference was found in cation excretion of Ca, Mg, Fe, and Zn in feces. No test-related gross pathologic lesions or variations in organ weight were observed at necropsy. No treatment-related histologic lesions were observed by microscopy of 31 organs examined. The NTA content in the bone increased in a dose-related manner. Histological examination of bone showed no differences in the amount of osteoid tissue or widths of the epiphyseal plates between samples from

control animals and animals of the high dose group. Because of increased urinary zinc excretion and unchanged fecal zinc excretion, a depletion of zinc was suggested by the authors. However, no signs of zinc deficiency (emaciation, emesis, conjunctivitis, keratitis, general debility and retardation of growth) were manifested in this study. Although urine zinc excretion was elevated, this was not accompanied by any renal lesions like in some rat studies.

Based on altered zinc excretion at doses from 0.14% Na₃NTA, the NOAEL of this feeding study in beagle dogs was estimated to be 0.03% Na₃NTA (11 mg/kg bw/d).

Drinking water studies:

Comment on the doses in oral drinking water studies on rats: If not otherwise mentioned, calculated intake of the test substance is based on an average water intake of 10% of the bw/d. If no data on the purity of the test substance is reported, 100% purity is assumed.

RAT- Subacute studies:

- In a comparative study on the concentrations of Na, K, Ca, Mg, P, S, Fe, Sr, Cu, and Zn in blood, plasma, brain, heart, muscle, liver, kidney, duodenum and bone of control rats and of rats (10 or 11 Wistar rats/ group) receiving Na₃NTA, EDTA or tripolyphosphate with the drinking water at a dose of 1 mmol/kg bw/d (appr. 257 mg Na₃NTA/kg bw/d) for 35 days (Krari and Allain, 1991).

Na₃NTA did not induce abnormal gross behavior, change of food intake and weight increase in comparison to the control group. The concentrations of the elements in the group treated with NTA inducing a significant decrease of the concentration of Mg in blood (-10%), of Cu in the liver (-9%), of S in the kidney (-7%), and of Ca (-33%), Mg (-18%), Fe (-31%), P (-22%), and Sr (-42%) in the duodenum. NTA increased the concentrations of Fe (+29%) in the liver and of Zn (+44%) in bone. No changes were detected in the muscle and the heart. The concentration of Zn in the kidney and plasma did not differ from that of the controls. The authors concluded that except for the increase of Zn in bone and Fe in liver induced by NTA there was no major changes of the tissue concentrations of elements.

RAT - Subchronic studies:

- In a drinking water study of 10 weeks duration Na₃NTA at either 0.01, 0.1 or 1% (w/v, appr. 10, 100, or 1000 mg/kg bw/d) was given to groups of 9 male Sprague-Dawley rats with or without lead (Mahaffey & Goyer, 1972). (Data on rats with lead cotreatment were discarded from this report.) At the end of the experiment, 24 hour urine samples were taken prior to the sacrifice. Kidneys were excised and weighed. Liver, brain, and pancreas were processed for histology. There was one early death in the mid dose group and a high mortality rate in the high dose group. Six animals died within four weeks, and remaining animals appeared moribund and were killed. No difference in total body weight or weight gain was observed in 0.01 and 0.1% levels of Na₃NTA in the drinking water after ten weeks of treatment. At 0.1% Na₃NTA the relative kidney weight was elevated. Histology of the organs examined did not differ from that of control animals. In contrast, kidneys from the 1% Na₃NTA group showed

marked vacuolization of renal tubules. Brain and pancreas of these animals were not examined microscopically, livers were normal. Urine volume of any of the Na₃NTA test groups did not differ from controls, but glycosuria was present in 5/7 of rats receiving 1% Na₃NTA. Mean fasting blood glucose levels were elevated in all groups receiving Na₃NTA comparing to control values. The NOAEL limited to the parameters of this test was 0.01% (appr. 10 mg/kg bw/d). The increased glucose levels at all doses were considered as treatment-related but not clearly adverse.

In a second experiment values of blood glucose after overnight fasting, blood urea nitrogen, and weight gain were investigated in another rat strain (Charles River CD, Sprague-Dawley derived?). Groups of 25 male rats received 0, 0.01, 0.05, 0.1% of Na₃NTA added to deionized drinking water for ten weeks. In the 0.1% Na₃NTA group two rats died before the end of the ten-week period. At 0.05% and 0.1% levels of Na₃NTA blood glucose levels are significantly elevated above control values. Blood urea nitrogen and weight gain did not differ among any of the groups. The NOAEL for effects on blood level on glucose was 0.01% Na₃NTA (appr. 10 mg/kg bw/d).

- Lower serum potassium were reported in an abstract of Becking and Yagminas (1978) in male rats treated with 100, 1000 or 5000 mg/l NTA (appr. 10, 100 or 500 mg/kg bw/d) in drinking water for up to 90 days. The α_2 and β globulin fractions in serum proteins were elevated in high dose animals. No significant changes in body weights, food and water intake or organ/body weight ratios were noted in all treated animals except higher liver/body weight and kidney/body weight ratios in animals given 5000 mg/l of NTA.

DOG - Chronic studies:

A 7-month drinking water administration of Na₃NTA at a concentration of 2.5 mg/kg bw/d to two male adult beagle dogs did not reveal any clinical, haematological or biochemical abnormalities (no data on test parameters examined, samples monthly taken) (Anderson & Danylchuk, 1980). In comparison to 22 control dogs of the same age, no significant difference of the plasma levels of parathormone was observed. A rib biopsy was taken before the commencement of the treatment at an age of 15 months and after 7 months of treatment. No altered zinc content was found in the bone at the end of treatment. A marginal decrease in the radial closure rate and in the percentage of osteoid seams taking a fluorescent label suggesting a minimal effect on the bone formation.

Animal studies with dermal administration:

RABBIT - Subacute studies:

- Two ml/kg/day of a 2.5% aqueous solution of Na₃NTA (\approx 50 mg/kg/day) was applied to the clipped and abraded backs (no data on cm² of skin area) of 6 New Zealand rabbits (2250-2900 g) for a period of 28 days (20 treatments) (Nixon, 1971). An additional group of 6 animals was treated with the same dose level applied daily to intact skin for a period of 91 days (65 treatments). Two ml of a 10% solution of granular detergent (no further details given) containing 10% Na₃NTA was also applied for 5 days each week to the intact skin of 6 albino rabbits for 91 days (65 treatments)

while a similar formulation containing 11% Na₃NTA was applied at the same dose level to a group of 6 animals abraded skin for 28 days (20 treatments). Animals with 2 ml/kg/day of water served as controls. Data on sex of animals and purity of the test substances were absent.

No adverse effects were observed grossly in any study. Growth, organ to body weight ratios (no data on organ weighed), and hematologic values were within the normal limits. Microscopic examination revealed that animals treated with the 10% and 11% Na₃NTA solutions showed only mild skin irritation, no treatment associated change was registered in any of the 15 internal organs examined.

Although no indication on systemic effects was observed, the systemic bioavailability of Na₃NTA is considered to be questionable, data reported were incompliant to standard test design and therefore a N/LOAEL for systemic toxicity can not be derived.

The NOAEL for local effects on the intact or abraded skin was 2.5% Na₃NTA in 2 ml solution (50 mg/kg bw/d).

Animal studies with intraperitoneal administration:

RAT - Subacute studies:

- Male Wistar rats, 7 weeks old, were injected i.p. either with 10 daily injections of saline (10 males), with 4 doses of Fe³⁺NTA (6 mg Fe/kg bw/d) or with equivalent doses of 75 mg NTA (NTA not further specified) over 6 days (Preece et al., 1989). In addition, another group received 9 doses of Fe³⁺NTA (5x6 mg Fe/kg bw/d and 4x12 mg Fe/kg bw/d) or NTA (5x75mg NTA/kg bw/d and 4x150 mg NTA/kg bw/d) over 13 days (6-10 rats, no exact data on no. of animals/group).

No effect on the mean organ to body ration for the liver and kidneys was seen in the NTA group. Administration of NTA alone had no effect on urine volume, creatinine or on the kidney brush-border enzymes, alkaline phosphatase or γ -glutamyl transpeptidase. No stainable iron was detected in control and NTA-treated groups. No NTA effect on haematology, hepatic function and urinary parameters was noted. However, NTA treatment was associated with a significant decrease in the iron concentration of the liver, but not of the kidney. Tissue copper and zinc concentrations of the liver and the kidney were unaffected in males after the 13-day treatment period with NTA.

The NOAEL for NTA in this study was derived to be 75 mg/kg bw/d.

Summary on repeated dose toxicity of NTA

Non-complexed NTA in its forms as Na₂NTA, Na₃NTA or H₃NTA was reported as NTA.

Animal studies with inhalation exposure

There were few data from two reports on inhalation studies in animals, both of which were incomplete compared to standard test protocols. NTA concentrations of 2 mg/l (6 h/d, 4 d) were irritative to the mucosa of the respiratory tract including the nose and the eyes of rats, whereas at 0.2 mg/l no adverse effect was reported (EPA, 1980). Prolongation of inhalation exposure to a period of 4 weeks (6 h/d, 5 d/wk) resulted in dyspnoe in rats and guinea pigs at NTA concentration of 0.34 mg/l, the NOAEC for both species was 0.21 mg/l (EPA, 1980). No adverse effect on the respiratory system was observed in monkeys at concentrations up to 0.34 mg/l NTA. Systemic effects associated to the inhalation of 0.34 mg/l NTA were diarrhea in monkeys and elevated ASAT activity and protein levels rats indicating a minor dysfunction of liver cell metabolism. The NOAEC of NTA for systemic effects was 0.34 mg/l in guinea pigs and 0.21 mg/l in rats and monkeys. Urinary tract toxicity was not reported for experimental animals of the above mentioned repeated dose inhalation studies (EPA, 1980), possibly because of methodical and documental insufficiencies of these studies. Since no laboratory or pathology data were available, no reliable value for the NOAEC for systemic effects could be estimated. Therefore the NOAEC_{systemic} of 0.21 mg/l (corresponds to 60.9 mg/kg bw/d) appears very uncertain.

Inhalation No Observed Adverse Effect Concentration (NOAEC)

NOAEC _{local} =	0.2 mg/l NTA	(rats & guinea pigs, 4 wk, 6 h/d, 5 d/wk) (EPA, 1980)
NOAEC _{systemic} =	0.2 mg/l NTA	(rats & monkeys, 4 wk, 6 h/d, 5 d/wk) (EPA, 1980)
(corresponds to 60.9 mg/kg/d (210 mg/m ³ NTA is multiplied by 0.29 m ³ /kg bw (rat breathing volume during 6 hours))		

Animal studies with oral administration

Rat studies with oral administration

Although NTA was tested in its different forms (Na₃NTA, Na₃NTA.H₂O, H₃NTA), the summary partly refers NTA as the active component for general consideration.

- NTA effects on the urinary tract

Target organs (*The main effects were also summarised in Tables 4.1.2.8.3.A, B*).

The organ system that was mainly affected by repeated oral treatment with NTA was the urinary tract with lesions at several sites: in the kidneys, ureters and urinary bladder (Alden et al., 1981; BASF, 1997b, 1998; Kanerva et al., 1984; Mahaffey & Goyer, 1972; Merski, 1982; Myers et al., 1982; Nixon, 1971; Nixon et al., 1972, see also 4.1.2.8 Carcinogenicity). Histomorphologic kidney lesions were discovered at several segments: the cortex area, the

renal papilla and the renal pelvis. The epithelium of the proximal convoluted tubules of the cortex region was found to be primarily affected. Another target tissue was the transitional cell epithelium (urothelium) of the renal pelvis, the ureters and the urinary bladder.

Gross and microscopic lesions

Gross findings after repeated administration were red-brown discoloured urine, enlarged kidneys, increased kidney to body ratio, discoloured kidneys, rough surface of the kidneys, hydronephrosis (pelvic dilation) and dilated ureters. One of the predominant microscopic lesions induced by NTA occurred primarily in the proximal tubules of the cortex region and was consistently reported as vacuolisation (of non-hyperplastic epithelial cells), degeneration of the tubular epithelium, and simple and nodular hyperplasia of the tubules. Tubular hyperplasia was reported to be frequently associated with vacuolisation of tubular cells (Merski, 1982; Myers et al., 1982). There also were basophilic (regenerative) hyperplasias without cellular vacuolation (BASF, 1998; Merski, 1982; Myers et al., 1982). Erosion/ulceration and hyperplasia of the transitional cell epithelium were observed in the renal pelvis (BASF, 1998). In studies with sequential sacrifices every fourth day, development of pelvic lesion started later and at a higher dose than tubular damage (Merski, 1982). After 7 weeks of NTA treatment, transitional cells also appeared to be dysplastic, and showed intracytoplasmic globules, and mitotic figures (Myers et al., 1982). Lesions at these two main loci may be associated to secondary responses as a sequelae to the epithelial cytotoxicity. They consisted of inflammation (interstitial or subepithelial), haemorrhage, fibrosis, dilatation of tubules and collection ducts, and tubular mineralisation of these lesions. Ultrastructurally, cellular lesions in the tubules were characterised as swelling, vacuolar degeneration of cytoplasmic organelles (endoplasmatic reticulum, mitochondria) and cell lysis (BASF, 1998).

The best insight in the cascade of histomorphologic events in the kidneys initiated by NTA treatment can be gained from the studies of Anderson et al. (1981) and of BASF (1998). Characteristic lesions reflected those described in Table 4.1.2.8.3 A.

Renal toxicity was observed in orally treated rats irrespective of the application modus using diet or drinking water containing NTA or gavage administration of NTA.

The minimal effective dose that induced toxicity in the urinary tract of rats was 0.15% Na₃NTA (97 mg/kg bw/d, see Table 4.1.2.6.2) and became evident in this study after treatment with diet on 6 months (Nixon et al., 1972).

Corresponding to the histomorphologic findings of kidney toxicity, the enzyme activities of LDH were increased in the urine of rats treated at 20000 ppm of NTA for 3 weeks (BASF, 1998). This enzyme is located in the tubular epithelial cells (distal>proximal) and at other organ sites. The increase may give indication on a tubular damage. No indication of a toxic effect on the papilla was found by the examination of N-acetyl- β -glucosaminidase, which was not altered by the treatment.

No indication on treatment-related accumulation of hemosiderin in kidneys was found at Na₃NTA.H₂O concentration of 20000 ppm (926 mg/kg bw/d) after a 4-week feeding period (BASF, 1998), macrophages laden with hemosiderin were distributed similarly in treated rats and control rats. In contrast, Nixon and his colleagues (1972) reported hemosiderin deposition in renal tubules at diet concentrations of 0.15% of Na₃NTA and above (\geq 97 mg/kg bw/d). Deposits may be considered to be remnants of cellular degradation following the degeneration of tubular epithelium. Alternatively they can be indicative for Fe-mobilisation from

endogenous sources. However, relationship to NTA treatment is uncertain due to the absence of this finding in other studies.

With respect to the regression of renal lesions, the incidence of basophilic hyperplasia in rats treated for 7 weeks with a diet containing 2% Na₃NTA.H₂O (1309 mg/kg bw/d) and a normal diet until the end of a 5-week recovery period remained increased (Myers et al., 1982). However, vacuolar degeneration of tubules and nodular forms of basophilic cell hyperplasia were not longer persistent after the recovery period. Additionally, a higher incidence of hydronephrosis persisted at the end of recovery.

Persistence of chronic renal inflammation was still seen after a treatment period of 18 months followed by 6 months of recovery (NCI, 1977; see 4.1.2.8 Carcinogenicity).

Thickening and erosion of the transitional cell epithelium of the ureters were also observed in rats (Kanvera et al., 1984). A dilation of the ureters was noted in one single rat out of four groups of five animals at 20000 ppm (926 mg/kg bw/d) (BASF, 1998).

Non-neoplastic changes the urinary bladder were not reported, probably because histopathologic examinations of the ureters and the urinary bladder were not routinely included in most of the repeated dose studies.

Urinalysis

Urine volume, specific gravity and pH-value:

NTA as Na₃NTA.H₂O and H₃NTA provoked inconsistent effects on the urinary volume and pH-value (Anderson & Kanerva, 1978, 1979). Na₃NTA.H₂O increased the urine pH-values (also Michael & Wakim, 1973) and increased the volume at doses of 525 mg/kg bw/d or higher (also BASF, 1998), both parameters were reversely changed after H₃NTA treatment. A dose-related reduction of urine pH-values were also reported for male rats at gavage doses of 150 mg/kg bw/d and higher and female rats at 1500 mg/kg bw/d (BASF, 1997b). Controversially, the urine volume was not changed after 10-weeks of drinking water exposure to concentrations up to 1% Na₃NTA (1000 mg/kg bw/d) (Mahaffey & Goyer, 1972).

Excretion of electrolytes and metals:

Different findings regarding the NTA-treatment related increase of electrolyte excretion with the urine were reported in the oral studies. Anderson & Kanerva (1978) reported a dose-dependently increase of Ca output at doses from 1540 mg/kg bw/d Na₃NTA.H₂O in a 3-week rat study. Rats receiving 1400 mg/kg Na₃NTA.H₂O for 30 days also showed elevated Ca concentrations (Michael & Wakim, 1973). It can be argued that Ca excretion may only be altered at very high doses, since no change on the Ca excretion was reported in the study of BASF (1998) at the highest dose tested (926 mg/kg bw/d, 4 week).

Urinary iron excretion was not altered by Na₃NTA.H₂O treatment for 4 weeks (BASF, 1998), whereas urinary Zn concentrations were increased in rats at 20000 ppm (926 mg/kg bw/d). Michael & Wakim (1973) reported higher Zn and Na excretion with the urine, but normal Fe concentrations in rats fed with 2% Na₃NTA.H₂O (1400 mg/kg bw/d) on 30 days.

Semiquantitative and microscopic examinations of the urine:

The sediment examination of the urine revealed conflicting results in rats with respect to excretion of crystals. Decreased amounts of crystals were observed in the 4-week study with Na₃NTA.H₂O doses at 20000 ppm (926 mg/kg bw/d, BASF, 1998), but crystalluria was seen after 3 and 4-week administration of doses from 525 mg/kg Na₃NTA.H₂O or H₃NTA (Anderson & Kanerva, 1978, 1979). Granular casts and tubular and transitional epithelial cells were observed in rat urine at a gavage dose of 1500 mg/kg bw/d of H₃NTA (BASF, 1997b). Transitional epithelial cells in the urine were transiently seen during a 4-week feeding period of 20000 ppm Na₃NTA.H₂O (926 mg/kg bw/d) (BASF, 1998).

Haematuria and elevated numbers of erythrocytes were seen in urine sediments of treated rats at 20000 ppm (926 mg/kg bw/d) Na₃NTA.H₂O or higher (BASF, 1998; Anderson & Kanerva, 1978) and at 1500 mg/kg bw/d of H₃NTA (BASF, 1997b).

Glucosuria indicative for reduced tubular reabsorption was found at 1% Na₃NTA (1000 mg/kg bw/d) given with the drinking water for 10 weeks (Mahaffey & Goyer, 1972).

Biochemical parameters indicative for renal damage:

Slightly higher serum urea levels, but no effect on creatinine concentrations were noted using strip methods in rats from a 3-week gavage study up to doses of 1500 mg/kg bw/d (BASF, 1997b). No quantitative urine analysis data on biomarkers of renal damage are available.

- Other NTA effects

Survival rates were reduced in male rats of a 2-year feeding study at a concentration of 0.5% Na₃NTA (322 mg/kg bw/d) (Nixon et al., 1972). Increased mortality rates were also seen in a 10-week drinking water study on rats receiving 1% Na₃NTA (1000 mg/kg bw/d), a single unscheduled death occurred at 0.1% Na₃NTA (100 mg/kg bw/d) (Mahaffey & Goyer, 1972). The mortality rates were also increased in male rats of a 2-year carcinogenicity study at diet concentrations of 20000 ppm NTA (921 mg/kg bw/d), but not at 2000 ppm NTA (92 mg/kg bw/d) (NCI, 1977). Single case of unscheduled deaths also occurred at NTA concentration of 20000 ppm in feed (926 mg/kg bw/d) after 28 days (BASF, 1998). Other studies did not reveal unscheduled mortalities.

Unspecific toxic effects due to subacute and subchronic administration of NTA were reduced body weight gain, lower feed consumption and feed efficiency at doses of 350 mg/kg bw/d Na₃NTA.H₂O, or 500 mg/kg bw/d H₃NTA and higher (BASF, 1997b, 1998; Anderson & Kanerva, 1978, 1979; Kanerva et al., 1984; Michael & Wakim, 1973; Myers et al., 1982; Nixon, 1971; NCI, 1977). The maximum doses of NTA without abnormalities on the growth was 150 mg/kg bw/d for 3 weeks (BASF, 1997b) and 322 mg/kg bw/d Na₃NTA in the first 8 weeks of a 2-year rat study (Nixon et al., 1972). Diarrhea, smeared anogenital region, perinasal encrustation and piloerection were observed in rats receiving 1500 mg/kg bw/d by gavage application (BASF, 1997b). Rats receiving 20000 ppm NTA in feed (926 mg/kg bw/d) showed increased water consumption and piloerection from day 21 on (BASF, 1998).

Mahaffey and Goyer (1972) reported that Na₃NTA in drinking water at concentrations of 0.01% to 1% (10-1000 mg/kg bw/d) induced elevated blood glucose levels in rats after treatment periods of 10 weeks, whereas BUN was unchanged. Animals treated at 20000 ppm Na₃NTA.H₂O (2000 mg/kg bw/d) showed reduced RBC counts and hemoglobin content indicating anemia (Nixon, 1971).

Higher liver to body ratios were estimated at diet concentrations of 20000 ppm (2000 mg/kg bw/d) Na₃NTA.H₂O for 90 days (Nixon, 1971) and at 0.5% Na₃NTA (322 mg/kg bw/d) after 12 months of (Nixon et al., 1972). Similarly, exposure to a concentration of 5000 mg/l NTA (500 mg/kg bw/d) in drinking water was also able to induce higher relative liver weights when administered to rats for 90 days (Becking & Yagminas, 1978). Oral Na₃NTA exposure increased Fe concentrations in the rat liver tissue (Krari and Allain, 1991).

Histopathology data on other organs outside the urinary tract were absent in most of the studies mentioned above. No lesions were observed in the liver, spleen and pancreas of rats after 4 week-treatments with 20000 ppm Na₃NTA.H₂O (926 mg/kg bw/d) (BASF, 1998). Liver lesions were not found in a drinking water study at 1% Na₃NTA (1000 mg/kg bw/d) (Mahaffey & Goyer, 1972). Nixon et al. (1972) did not report histopathologic abnormalities in 15 organs in rats of a 90-day feeding study with doses up to 20000 ppm Na₃NTA.H₂O (2000 mg/kg bw/d).

Although a low percentage of NTA was deposited in the skeleton (Michael and Wakim, 1971; Tjälve, 1972), no clear adverse effect of NTA treatment on the bone was seen of rats in a 2-year feeding study at concentrations of Na₃NTA (322 mg/kg bw/d) (Nixon et al., 1972). The morphology and breaking strength of the femur appeared to be normal; the only finding was NTA deposition in the bone matrix. Increased deposition of Zn was associated to NTA treatment in rats receiving appr. 239 mg/kg bw/d Na₃NTA within drinking water on 35 days (Krari & Allain, 1991).

No indication on relevant alteration of electrolyte homeostasis except higher Zn concentration in bone and Fe concentration in liver was demonstrated in rats that were orally administered to 1 mmol/kg/d NTA (191 mg/kg bw/d) for 35 days (Krari & Allain, 1991). No major difference was seen in the concentration of Na, K, Mg, P, S, Fe, Sr, Cu, and Zn in blood, plasma, brain, heart, muscle, kidney and duodenum.

There were some indications of altered protein metabolism. Becking & Yagminas (1978) reported elevated α_2 and β globulin fractions in serum proteins of rats exposed via the drinking water to 5000 mg/l NTA (500 mg/kg bw/d). Increased serum concentrations of albumin, globulin, total protein and gamma globulin were seen in rats after inhalation to 0.34 mg/l NTA for 4 weeks (EPA, 1980).

Oral studies in other species

In mice, a 20-week feeding of 1% Na₃NTA.H₂O or H₃NTA induced elevated values of BUN and liver enzymes (LDH, ASAT, ALAT) (Matsuki et al., 1992). No altered urinary concentrations of Zn, Cu, Mg, Mn, Fe, and Ca were determined in daily urine from mice injected i.p. 100 mg/kg bw/d NTA for 3 consecutive days (Cantilena & Klaassen, 1982).

Dogs receiving Na₃NTA on 90 days with feed presented a dose-related increase of NTA content in their bones and a highly variable, but increased urinary Zn excretion (≥ 42.5 mg/kg bw/d), however no changes of hematology and clinical chemistry parameters, serum concentrations of Ca, Mg, Na, Fe, Zn, Co, inorganic phosphorus, urinary concentrations of Ca, Mg, Na, Fe, and K and fecal concentrations of Ca, Mg, Fe, and Zn at doses up to 130 mg/kg bw/d (Budny et al., 1973). No treatment-related abnormality in gross pathology or histopathology was found in any of 31 organs/tissues (including the kidneys, urinary bladder and bone) at diet concentrations up to 0.5% Na₃NTA (130 mg/kg bw/d).

A low dose of 2.5 mg/kg bw/d Na₃NTA applicated with the drinking water for 7 months to dogs did not alter bone Zn content (Anderson & Danylchuk, 1980).

Oral No / Lowest observed adverse effect level (N/LOAEL)

From all oral studies mentioned above, those studies were considered for the derivation of an overall N/LOAEL that at minimal included the examination of toxic effects on the urinary tract. Some other studies focussed only on very specific questions like the electrolyte status and therefore were excluded. The L/NOAEL was derived for NTA-related toxicity in target organs. Hyperplasias were presumed for being preneoplastic changes and were taken into consideration in Section 4.1.2.8 Carcinogenicity.

For overview, Table 4.1.2.6.2 may be helpful to identify the highest NOAEL for long-term toxicity tested, which was below the lowest LOAEL (grey bars in Table 4.1.2.6.2) identified. For further risk assessment procedures on noncancer endpoints, the NOAEL of 92 mg derived from the 24 month cancer study (NCI, 1977) could be proposed. Attention should be paid to that this NOAEL for long-term toxicity is very close to the lowest dose demonstrated to be carcinogenic in rats, which is 1% (100 mg/kg bw/d) Na₃NTA (Goyer et al., 1981). Also, the NOAEL for long-term toxicity is identical to the dose at those preneoplastic lesions in rats were increased after receiving NTA for 24 months with diet (NCI, 1977).

Comparing the effect level for non-cancer and preneoplastic/cancer endpoints (see 4.1.2.8) indicates that urinary tract carcinogenicity was more sensitive than urinary tract toxicity. Urinary tract toxicity was considered as a precondition in the development of preneoplastic changes and at final of tumors. Unlike commonly expected cytotoxicity was not the most sensitive effect; since hyperplasia was observed at lower doses than cytotoxicity (see discussion on Strength and consistency in 4.1.2.8.3). Therefore it is proposed to use the BMD calculated for carcinogenicity (see 4.1.2.8.4) rather than to apply the NOAEL for cytotoxicity as a solitary effect for risk assessment on repeated dose toxicity.

Table 4.1.2.6.1 No/Lowest-observed adverse effect levels of NTA toxicity from oral repeat-dose studies on rats and mice

NOAEL		LOAEL		Study design	Reference
NTA species / diet concentration*	dose (mg/kg bw/d)	NTA isoform/ concentration	diet dose (mg/kg bw/d)		
Subacute gavage studies					
H ₃ NTA	150	H ₃ NTA	500	rat, 3 wk	BASF, 1997b
	not estimated	Na ₃ NTA.H ₂ O 0.73 mmol/kg bw/d	188	rat, 30 d	Merski, 1982
Subacute/subchronic feeding studies					
Na ₃ NTA:H ₂ O 150 ppm	9	Na ₃ NTA.H ₂ O 20000ppm	926	rat, 4 wk	BASF, 1998
	not estimated	Na ₃ NTA.H ₂ O 0.5%	350	rat, 4 wk	Anderson & Kanerva, 1978
	not estimated	H ₃ NTA 0.5%	350	rat, 4 wk	Anderson & Kanerva, 1978
	not estimated	Na ₃ NTA.H ₂ O 2%	1400	rat, 4 wk	Anderson & Kanerva, 1979
	not estimated	H ₃ NTA 1.5%	1050	rat, 4 wk	Anderson & Kanerva, 1979
	not estimated	Na ₃ NTA.H ₂ O 2%	1400	rat, 4 wk	Kanerva et al., 1984
	not estimated	Na ₃ NTA 2%	1400	rat, 30 d	Michael & Wakim, 1973
	not estimated	Na ₃ .NTA.H ₂ O 2%	1309	rat, 7 wk	Myers et al., 1982
	not estimated	H ₃ NTA 1.5%	1050	rat, 7 wk	Myers et al., 1982

NOAEL		LOAEL		Study design	Reference
Na ₃ NTA 2000 ppm	200	Na ₃ NTA 20000 ppm	2000	rat, 90 d	Nixon, 1971
Subacute/subchronic drinking water studies					
Na ₃ NTA 0.01%	10	Na ₃ NTA 0.1%	100	rat, 10 wk	Mahaffey & Goyer, 1972
Combined chronic toxicity/cancer feeding studies					
Na ₃ NTA 0.03%	19	Na₃.NTA 0.15%	97	rat, ≥6 mo	Nixon et al., 1972
Na₃NTA.H₂O 2000 ppm	92	Na ₃ NTA.H ₂ O 20000ppm	921	rat, 24 mo	NCI, 1977
	Not estimated	NTA 7500 ppm	526	rat, 18 mo + 6 mo rec	NCI, 1977
	Not estimated	Na ₃ NTA.H ₂ O 7500 ppm	355	rat, 18 mo + 6 mo rec	NCI, 1977
	Not estimated	NTA 7500 ppm	752	mouse, 18 mo + 3 mo rec	NCI, 1977
Na ₃ NTA.H ₂ O 2500 ppm	169	NTA 5000 ppm	338	mouse, 18 mo + 3 mo rec	NCI, 1977
Cancer drinking water studies					
Na ₃ NTA 0.1%	100	Not estimated		rat, 704 d	Goyer et al., 1981

* if not otherwise indicated % or ppm in diet or drinking water, grey bars indicating the lowest value of the LOAEL and the NOAEL next below this value.

Animal studies with dermal exposure

A Na₃NTA concentration without irritative effects was 2.5% Na₃NTA aqueous solution (2 ml, ≈ 50 mg/kg bw/d) represented the NOAEL for local effects on the rabbit dermis. Systemic effects were not observed in this 90-day study (Nixon, 1971). Because of incomplete data reported, the study should not be accepted for derivation of a systemic NOAEC.

DERMAL NO OBSERVED ADVERSE EFFECT CONCENTRATION

NOAEC _{local} =	2.5% Na ₃ NTA	(50 mg/kg/d), (rabbit, 90 d, 5d/wk) (Nixon, 1971)
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NOAEC _{systemic} =	not estimated for the dermal route	
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Animal studies using intraperitoneal application

9 doses of 150 mg/kg bw/d NTA in a period of 13 days were not able to induce differences neither in gross findings, haematological, biochemistry and urinary parameters nor in the enzymes of the tubular brush-border of rats. The only finding associated with NTA treatment was a decrease of Fe-content in the liver (Preece et al., 1989).

Rats injected for 4 weeks intraperitoneally with appr. 16.8 mg/kg bw/d of NTA at four weeks after partial nephrectomy or sham-operated showed increased tubular damage accompanied with proteinuria, increased serum creatinine levels.

4.1.2.7 Mutagenicity

In the following effect assessment on mutagenicity of trisodium nitrilotriacetic acid (Na₃NTA) besides Na₃NTA itself also data on Na₃NTA.H₂O, Na₂NTA and NTA are considered.

The majority of in vitro tests with mammalian cell cultures were conducted only without S-9 mix. Since no biotransformation of NTA is known, this does not necessarily discount the tests.

4.1.2.7.1 In vitro tests: Bacterial genotoxicity

Bacterial genotoxicity tests are available for trisodium nitrilotriacetic acid monohydrate (Na₃NTA.H₂O), disodium nitrilotriacetic acid (Na₂NTA) and nitrilotriacetic acid (NTA).

Na₃NTA.H₂O was negative with respect to the induction of bacterial gene mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WPuvrA (Dunkel et al., 1985; Zeiger et al., 1992; Loprieno et al., 1985). Using the pre-incubation methodology Na₃NTA.H₂O was tested without S-9 mix and in presence of S-9 mix obtained from rat, mouse or Syrian hamster livers in doses ranging from 3.0 to 10000 µg/plate (Dunkel et al., 1985; Zeiger et al., 1992). In the standard plate assay

doses up to 870 µg/ml were tested in absence and presence of standard rat liver S-9 mix (Loprieno et al., 1985). In all these studies data on toxicity were not given.

Negative results in various *Salmonella typhimurium* tester strains were also obtained with Na₂NTA (Nakatsuka et al., 1989; doses up to 600 µg/plate) and NTA (Zeiger et al., 1992; doses up to 2000 µg/plate).

According to Venier et al. (1989) 'NTA' (no further specification) did not induce bacterial SOS repair in the SOS chromotest using *E. coli* strain PQ37 with and without S-9 mix in doses up to 1000 µg/test.

It can be summarised that the sodium salts of NTA and NTA were negative in bacterial genotoxicity tests.

Table 4.1.2.7.1. In vitro tests: Overview on bacterial genotoxicity

Test system	Concentration range		Result	Toxicity	Test substance	Reference
	with S-9 mix	without S-9 mix				
Gene mutations, Salm. typh. strains	3 - 3333 µg/plate	3 - 3333 µg/plate	negative	no data	Na ₃ NTA.H ₂ O	Dunkel et al., 1985
Gene mutations, Salm. typh. strains	100 - 10000 µg/plate	100 - 10000 µg/plate	negative	no data	Na ₃ NTA.H ₂ O	Zeiger et al., 1992
Gene mutations, Salm. typh. strains	54 - 870 µg/plate	54 - 870 µg/plate	negative	no data	Na ₃ NTA.H ₂ O	Loprieno et al., 1985
Gene mutations, Salm. typh. strains	not done	1 - 600 µg/plate	negative	no data	Na ₂ NTA	Nakatsuka et al., 1989
Gene mutations, Salm. typh. strains	33 - 2000 µg/plate	33 - 2000 µg/plate	negative	no data	NTA	Zeiger et al., 1992
SOS chromotest, <i>E. coli</i>	0.01 - 1000 µg/test	0.01 - 1000 µg/test	negative	no data	'NTA'	Venier et al., 1989

4.1.2.7.2 In vitro tests: Chromosomal aberrations and micronuclei

In vitro tests for induction of chromosomal aberrations or micronuclei are available for trisodium nitrilotriacetic acid (Na₃NTA), trisodium nitrilotriacetic acid monohydrate (Na₃NTA.H₂O) and nitrilotriacetic acid (NTA).

No well-conducted routine investigation is available. Nevertheless, three investigations can be used for risk assessment in spite of minor methodological problems (Modesti et al., 1995;

Loveday et al., 1989; Robbiano et al., 1989). Further 4 investigations are not used for further risk assessment because of severe methodological insufficiencies.

Modesti et al. (1995) investigated the effect of Na₃NTA on the induction of chromosomal anomalies ('chromatin bridges and acentric fragments' and lagging chromosomes) and micronuclei in Chinese hamster Cl-1 cells in the absence of S-9 mix. Cells were treated with doses ranging from 0.6 to 3.0 mmol/l for 24 or 48 h. At doses from 2 mmol/l upwards weak increases were found in the frequencies of micronuclei and chromosomal anomalies; for micronuclei the maximum effect was an increase by a factor of about 2.5 as compared to the negative control. Toxicity data were not given.

In a screening assay with CHO cells, NTA was negative for induction of chromosomal aberrations with and without S-9 mix for doses of 0.5, 1.5 or 5.0 µg/ml (equivalent to 2.6, 7.8 or 26.2 µmol/l; Loveday et al., 1989). Only 1 experiment was performed with 100 analysed metaphases per experimental entry. With S-9 mix exposure was for 2 h with 10 h recovery, without S-9 mix exposure was for 8 h with 2.0 to 2.5 h recovery.

NTA induced micronuclei in primary cultures of rat and human kidney cells (Robbiano et al., 1999). In both cell types ca. 2-fold increases were found at 1800 µmol/l and ca. 3-fold increases at 5600 µmol/l (negative control, 0.18% for both cell types); a slight decrease in cell survival was observed for the highest concentration. The tests were run without S-9 mix and without use of cytochalasin B, exposure for 48 h was followed by a 48-h recovery period.

As mentioned above there are 4 more investigations on cytogenetic effects of NTA in mammalian cell cultures, which are not adequate for risk assessment due to severe methodological insufficiencies.

The effect of Na₃NTA on chromosomal aberration frequencies in human lymphocytes without S-9 mix was investigated by Montaldi et al. (1988). After 2 days pre-incubation followed by 1 day of treatment with NTA no induction of chromosomal aberrations was observed for concentrations ranging from 1 to 5 mmol/l. Concentrations of 7.5 and 10 mmol/l were too toxic for chromosomal analysis. It has to be noted that reduced numbers of metaphases were scored for some experimental points; it may be assumed that the quality of metaphase preparations was not adequate; furthermore, the analysis was done without coding of the slides. Additionally two unusual time schedules were used: 2 day pre-incubation followed by 5 days treatment and 4 days incubation followed by 3 days treatment. Since no positive effects can be expected with these designs and an unusual high frequency of aberrant cells (5.5%, excl. gaps) was found in negative controls, these results are not meaningful.

Montaldi et al. (1987) reported on a screening chromosomal aberration test with Na₃NTA using human lymphocyte cultures in absence of S-9 mix. With 24 h treatment time no induction of structural chromosomal aberrations was observed for doses ranging from 2 to 6 mmol/l. Longer treatments for 48 and 72 h also resulted in negative findings. Only one experiment was performed and no positive control was employed. In the same investigation Na₃NTA was negative for induction of micronuclei in doses up to 10 mmol/l (used as negative control for combination experiments NTA + metals; no use of cytochalasin B). These data suffer from unusual low frequencies of micronucleated cells (ca. 0.2 to 0.3%), an extremely long treatment time of 72 h, and the lack of toxicity data.

Kihlman and Sturelid (1970) analysed chromosomal aberration frequencies in non-routine cells (rat kangaroo PT K1 cells) without S-9 mix after treatment with Na₃NTA.H₂O in doses ranging from 2.5 to 10 mmol/l. Weak increases in aberration frequencies were found at treatment times 24 and 48 h, the maximum effect was 5.3 chromatid breaks and 4.7 isochromatid breaks per 100 cells (no aberrations of these types were found in the negative control). 4-h treatment was negative for all doses. Only one experiment was performed, a positive control is lacking, toxicity data are not given.

Summary for chromosomal aberrations and micronuclei in vitro: No well-conducted routine test on chromosome aberration or micronuclei according to the present state of the art is available. From the data presented it may be concluded that Na₃NTA has a weak potential for induction of chromosomal aberrations/micronuclei in vitro.

Table 4.1.2.7.2. In vitro tests: Overview on chromosomal aberration and micronucleus tests

Test system	Concentration range		Result	Toxicity	Remarks	Test subst.	Reference
	with S-9 mix	without S-9 mix					
Chrom. anomalies and micronuclei, CI-1 cells	not done	0.6 - 3.0 mmol/l	positive	no data	weakly positive	Na ₃ NTA	Modesti et al., 1995
Chrom. aberrat., CHO cells	2.6 - 26.2 µmol/l	2.6 - 26.2 µmol/l	negative	no data	screening procedure	NTA	Loveday et al., 1989
Micronuclei, primary cultures of rat & human kidney cells	not done	1800 - 5600 µmol/l	positive	slight toxicity at 5600 µmol/l	clear increases in both cell types	NTA	Robbiano et al., 1999
Chrom. aberrat., human lymph.	not done	1 - 7.5 mmol/l	negative	clear toxic at high doses	inadequate methodology	Na ₃ NTA	Montaldi et al., 1988
Chrom. aberrat., human lymph.	not done	2 - 4 mmol/l	negative	clear toxic at high doses	inadequate methodology	Na ₃ NTA	Montaldi et al., 1987
Micronuclei, human lymph.	not done	0.1 - 10 mmol/l	negative	no data	inadequate methodology	Na ₃ NTA	Montaldi et al., 1987
Chrom. aberrat., rat kangaroo cells	not done	2.5 - 10 mmol/l	inconclusive	no data	inadequate methodology	Na ₃ NTA.H ₂ O	Kihlmann and Sturelid, 1970

4.1.2.7.3 In vitro tests: Mammalian cell gene mutations

Mammalian cell gene mutation tests are available for trisodium nitrilotriacetic acid (Na₃NTA) and trisodium nitrilotriacetic acid monohydrate (Na₃NTA.H₂O).

Two mouse lymphoma assays with Na₃NTA led to negative results (Mitchell et al., 1988; Myhr and Caspary, 1988). In both investigations two experiments were performed each with and without S-9 mix, treatment was for 4 h. With S-9 mix doses ranging from 125 to 3000 µg/ml (0.5 to 11.7 mmol/l) were tested, without S-9 mix doses from 125 to 1900 µg/ml (0.5 to 7.4 mmol/l). With and without S-9 mix the highest tested doses led to strong cytotoxic effects.

Also in an HPRT test with V79 cells a negative result was obtained with Na₃NTA after 24 h exposure to 0.1 to 15 mmol/l (Celotti et al., 1987). This test was only run without S-9 mix, the highest dose led to clear cytotoxic effects.

Na₃NTA.H₂O was tested in a non-routine system with human EUE cells in which resistance to diphtheria toxin was analysed in the absence of S-9 mix (Grilli and Capucci, 1985). Doses ranging from 0.01 to 11 mmol/l led to increased mutation frequencies after 24 h treatment (13.5 to 30.5 mutants per 10⁶ cells, negative control 1.7 mutants); the highest dose was in the cytotoxic range. Only one experiment was performed. This test result is extremely difficult to interpret.

Summary of mammalian cell gene mutation tests: Na₃NTA was negative in well-conducted mouse lymphoma assays with and without S9 mix and in an HPRT test without exogenous metabolic activation. In a non-routine gene mutation test a positive result was obtained in one experiment. Overall, it is concluded that the tested NTA salts have no potential for induction of gene mutations in mammalian cells.

Table 4.1.2.7.3. In vitro tests: Overview on mammalian cell gene mutation tests

Test system	Concentration range		Result	Toxicity	Remarks	Test subst.	Reference
	with S-9 mix	without S-9 mix					
Mouse lymphoma assay	2.4 - 9.1 mmol/l	2.0 - 7.4 mmol/l	negative	strong toxic at highest dose	4 h treatment	Na ₃ NTA	Mitchell et al., 1988
Mouse lymphoma assay	0.5 - 11.7 mmol/l	0.5 - 4.7 mmol/l	negative	strong toxic at highest dose	4 h treatment	Na ₃ NTA	Myhr and Caspary, 1988
HPRT test, V79 cells	not done	0.1 - 15 mmol/l	negative	strong toxic at highest dose	24 h treatment	Na ₃ NTA	Celotti et al., 1987
Resistance to diphtheria toxin, EUE cells	not done	0.002 - 11 mmol/l	positive	strong toxic at highest dose	non-routine system	Na ₃ NTA.H ₂ O	Grilli and Capucci, 1985

4.1.2.7.4 In vitro tests: Sister chromatid exchanges (SCE)

In vitro tests for induction of sister chromatid exchanges (SCE) are available for trisodium nitrilotriacetic acid (Na₃NTA), trisodium nitrilotriacetic acid monohydrate (Na₃NTA.H₂O), disodium nitrilotriacetic acid (Na₂NTA) and nitrilotriacetic acid (NTA).

Na₃NTA, Na₃NTA.H₂O, Na₂NTA or NTA gave negative results when tested in absence of S-9 mix in CHO cells (Montaldi et al., 1985; Brat and Williams, 1984; Loveday et al., 1989), in V79 cells (Hartwig et al., 1993), in human lymphocytes (Brat and Williams, 1984) or in mouse lymphocytes (Montaldi et al., 1985). Doses up to 2 mmol/l were tested, toxicity data were not given in any of these studies.

In CHO cells NTA was also analysed in presence of S-9 mix (Loveday et al., 1989). In a screening assay low doses up to 26.2 µmol/l were negative, toxicity data were not described.

Summary of SCE tests: Sodium salts of NTA and NTA were negative in SCE tests with various cell types (in general, without S-9 mix).

Table 4.1.2.7.4. In vitro tests: Overview on tests for induction of sister chromatid exchanges (SCE)

Test system	Concentration range		Result	Toxicity	Remarks	Test subst.	Reference
	with S-9 mix	without S-9 mix					
CHO cells, mouse lymph.	not done	1 µmol/l - 2 mmol/l	negative	not given		Na ₃ NTA	Montaldi et al., 1985
CHO cells	not done	1 µmol/l - 1 mmol/l	negative	not given	screening assay	Na ₃ NTA.H ₂ O	Brat and Williams, 1984
V79 cells	not done	0.5 - 1.5 mmol/l	negative	not given		Na ₂ NTA	Hartwig et al., 1993
CHO cells	2.6 - 26.2 µmol/l	2.6 - 26.2 µmol/l	negative	not given	screening assay	NTA	Loveday et al., 1989
Human lymph.	not done	1 µmol/l - 1 mmol/l	negative	not given		NTA	Brat and Williams, 1984

4.1.2.7.5 In vitro tests: Unscheduled DNA synthesis (UDS)

In vitro tests for induction of unscheduled DNA synthesis (UDS) are available for trisodium nitrilotriacetic acid monohydrate (Na₃NTA.H₂O) and a non-specified 'sodium salt of NTA'.

Na₃NTA.H₂O did not induce DNA repair in the routine autoradiography test for unscheduled DNA synthesis (UDS) with primary cultures of rat hepatocytes. In two investigations doses ranging from 1.6 to 3.6 mmol/l were tested (Williams et al., 1982; 1989).

Celotti et al. (1988) investigated the effect of a non-specified 'sodium salt of NTA' on UDS in human lymphocyte cultures with an autoradiography as well as with a liquid scintillation methodology. 21 h after stimulation with phytohemagglutinin lymphocytes were treated for 3 h with the test substance in doses ranging from 5 to 10 mmol/l. According to the authors there was a dose-dependent positive effect; however, rather weak increases were found together with an extreme variation between replicates. Furthermore, cells were co-exposed to 2.5 mmol/l hydroxyurea in order to inhibit replicative DNA synthesis. Altogether, the result is evaluated as inconclusive.

Summary of UDS tests: Sodium salts of NTA were negative in routine UDS tests with primary rat hepatocytes; in human lymphocytes without S-9 mix an inconclusive result was found.

Table 4.1.2.7.5. In vitro tests: overview on tests for induction of unscheduled DNA synthesis (UDS)

Test system	Concentration range		Result	Toxicity	Remarks	Test substance	Reference
	with S-9 mix	without S-9 mix					
Primary rat hepatocytes	not applicable	1.6 - 3.2 mmol/l	negative	no data	autoradiography	Na ₃ NTA.H ₂ O	Williams et al., 1982
Primary rat hepatocytes	not applicable	3.6 mmol/l	negative	no data	autoradiography; screening procedure	Na ₃ NTA.H ₂ O	Williams et al., 1989
Human lymphocytes	not applicable	5 - 10 mmol/l	inconclusive	no data	co-exposure to hydroxyurea; weak increase, extreme variation between replicates; autoradiography and liquid scintillation counting	'sodium salt of NTA'	Celotti et al., 1988

4.1.2.7.6 In vitro tests: DNA strand breaks

In vitro tests for induction of DNA strand breaks are available for disodium nitrilotriacetic acid (Na₂NTA) and nitrilotriacetic acid (NTA).

In a dose of 1000 µmol/l Na₂NTA did not induce DNA strand breaks in V79 cells without S-9 mix as measured by the nucleosid sedimentation methodology (Hartwig et al., 1993). Treatment was performed for 1 to 24 h and did not decrease the colony forming ability of the cells.

In primary cultures of rat and human kidney cells DNA strand breaks were induced by NTA as measured in the so-called comet assay (Robbiano et al., 1999). All tested doses of 1800, 3200 and 5600 µmol/l led to increased damage of nuclear DNA (tail length). However, the observation of effects was associated with extremely high standard deviations, e.g. in human cells treated with a dose of 5600 µmol/l NTA a tail length of 17.1 +/- 12.4 µm was observed with a negative control of 6.8 +/- 8.3 µm. The maximum effect was found in rat cells after treatment with 5600 µmol/l NTA (322.7 +/- 18.8 µm as compared to an overall negative control of 3.6 +/- 3.2 µm). The tests were run without S-9 mix, exposure was for 20 h (without recovery).

Summary of DNA strand break tests: High doses of NTA induced DNA strand breakage in primary cultures from rat or human kidney cells.

Table 4.1.2.7.6 In vitro tests: overview on tests for induction of DNA strand breaks

Test system	Concentration range		Result	Toxicity	Remarks	Test substance	Reference
	with S-9 mix	without S-9 mix					
V79 cells	not done	1 mmol/l	negative	non-toxic		Na ₂ NTA	Hartwig et al., 1993
Primary cultures of rat & human kidney cells	not done	1800 - 5600 µmol/l	positive	slight toxicity at 5600 µmol/l	clear increases in both cell types	NTA	Robbiano et al., 1999

4.1.2.7.7 In vitro tests: Non-routine tests with other eukaryotic cells

Non-routine assays with lower eukaryotic organisms are available for trisodium nitrilotriacetic acid (Na₃NTA), trisodium nitrilotriacetic acid monohydrate (Na₃NTA.H₂O) and nitrilotriacetic acid (NTA).

From assays with lower eukaryotes negative results were obtained with and without S-9 mix for gene mutations in *Schizosaccharomyces pombe* P1 and for gene conversion in *Saccharomyces cerevisiae* D4 (Na₃NTA.H₂O; Loprieno et al., 1985). Without S-9 mix negative results were reported for gene mutations and chromosome malsegregation in *Aspergillus nidulans* (NTA; Crebelli et al., 1986) and SCE in developing eggs of *Mytilus galloprovincialis* (NTA; Brunetti et al., 1986).

In plant root tip systems without S-9 mix, induction of chromosomal aberrations or micronuclei by Na₃NTA (or its monohydrate) was analysed in two investigations.

Kihlman and Sturelid (1970) screened doses ranging from 5 to 20 mmol/l in root tips of beans for induction of chromosomal aberrations and obtained an inconclusive result. A weak increase was found for 5 mmol/l and 24 h treatment; 2 h exposure was negative for all doses. Only one experiment was performed, toxicity was not measured.

Marco et al. (1986) analysed micronuclei in root tips from *Vicia faba* and *Allium cepa*. After continuous treatment for 24 to 72 h doses of 2 to 4 mmol/l were positive in *Vicia faba* and 4 mmol/l was positive in *Allium cepa*.

Summary of non-routine assays with lower eukaryotes: In general, negative results were obtained in various test systems. However, in plant root tips there is evidence for induction of chromosomal effects in high doses.

Table 4.1.2.7.7 In vitro tests: Overview on non-routine tests with lower eukaryotes

Test system	Concentration range		Result	Test substance	Reference
	with S-9 mix	without S-9 mix			
Gene mutat., <i>Schizosaccharomyces pombe</i>	8 - 128.6 µmol/l	8 - 128.6 µmol/l	Negative	Na ₃ NTA.H ₂ O	Loprieno et al., 1985
Gene conversion, <i>Saccharomyces cerevisiae</i>	8 - 128.6 µmol/l	8 - 128.6 µmol/l	Negative	Na ₃ NTA.H ₂ O	Loprieno et al., 1985
Chrom. malsegregation & gene mutat., <i>Aspergillus nidulans</i>	not done	1.8 - 72 mmol/l	Negative	NTA	Crebelli et al., 1986
SCE, <i>Mytilus galloprovincialis</i>	not done	26.2 mmol/l	Negative	NTA	Brunetti et al., 1986
Chrom. aberrat., plant root tips	not done	5 - 20 mmol/l	Inconclusive	Na ₃ NTA.H ₂ O	Kihlman and Sturelid, 1970
Micronuclei, plant root tips	not done	1 - 4 mmol/l	Positive	Na ₃ NTA	Marco et al., 1986

4.1.2.7.8 In vitro tests: Cell transformation

Cell transformation tests are available for trisodium nitrilotriacetic acid (Na₃NTA).

Three different types of cell transformation assays were conducted, all of them without exogenous metabolism.

The only routine test was performed with mouse C3H/10T1/2 cells (Dunkel et al., 1988). Two laboratories were included in this investigation. In one lab a positive result was found for doses ranging from 2 to 250 µg/ml (7.8 to 973 µmol/l) without dose-dependency, the other

lab obtained a negative result for doses up to 100 µg/ml (389 µmol/l). All doses were associated with relatively low toxicity. All in all, the outcome is inconclusive.

A transformation test with baby hamster kidney cells (BHK cells) was negative for doses up to 10 mmol/l (Lanfranchi et al., 1988); however, this test system (Styles test) is of relatively low reliability.

Traul et al. (1981) tested Na₃NTA in rat embryo cells which were infected with Rauscher leukemia virus. Doses were given as 495 and 540 µg per 52000 cells (equivalent to LD₂ to LD₄₀). After 3 days treatment and 6 days recovery, analysis was done by comparing cell counts in treated cultures with those in negative controls. For both doses the increase in cell count over control was 96%. The authors state that the criterion for a positive finding (85% increase) was met. However, there is no plausible justification of this criterion. Furthermore, the reliability of cell count as indicator of cell transformation is doubtful. Therefore, the finding is evaluated as inconclusive.

Summary on cell transformation tests: No reliable data are available.

Table 4.1.2.7.8 In vitro tests: overview on cell transformation tests

Test system	Concentration range		Result	Remarks	Test substance	Reference
	with S-9 mix	without S-9 mix				
C3H/10T1/2 cells	not done	7.8 - 973.3 µmol/l	inconclusive	Positive in one lab, negative in another	Na ₃ NTA	Dunkel et al., 1988
BHK cells	not done	2 - 10 mmol/l	negative		Na ₃ NTA	Lanfranchi et al., 1988
Virus infected rat embryo cells	not done	not given	inconclusive	no plausible criteria for a positive result	Na ₃ NTA	Traul et al., 1981

4.1.2.7.9 In vivo tests: Rodent bone marrow tests

Rodent bone marrow genotoxicity tests are available for trisodium nitrilotriacetic acid (Na₃NTA).

In a micronucleus assay on polychromatic erythrocytes with male NMRI mice, Na₃NTA led to a negative result after two oral administration of 500, 1000 or 2000 mg/kg (BASF, 2004). The gavage treatments were 24 h apart; sampling was 24 h after the 2nd treatment. There were no clinical signs of toxicity and no local cytotoxicity in the bone marrow (ratio polychromatic vs. normochromatic erythrocytes).

In a further micronucleus test on polychromatic erythrocytes with male mice, conducted according to a screening procedure, single intraperitoneal administration of 200 or 400 mg/kg Na₃NTA led to an inconclusive finding (Montaldi et al., 1988). At all sampling times of 6, 12 and 24 h, the dose 200 mg/kg was clearly negative, and with 400 mg/kg for all sampling times

approximately a doubling of micronucleus frequencies was found (as compared to the negative control) without statistical significance. Three animals per dose group were used; data on general toxicity were not given, there was no effect on local cytotoxicity.

Russo et al. (1989) found a negative effect of Na₃NTA on aneuploidy in proliferation of bone marrow cells of male mice. 2 h after subcutaneous implantation of BrdUrd tablets 3 to 5 animals were treated once with 138 or 275 mg/kg (0.54 or 1.07 µmol/kg) by the intraperitoneal route. Analysis of hyperhaploidy was done in 300 to 555 second metaphases per group (identified by differential staining). Treatments had no influence on average generation times of the target cells; data on general toxicity were not given. In the same animals and target cells there was no induction of SCE which were analysed in 25 metaphases per animal. Due to the small numbers of animals and cells analysed, especially the result of the aneuploidy test is of low reliability.

Summary of rodent bone marrow tests: In a rodent bone marrow micronucleus test Na₃NTA was negative. In screening assays Na₃NTA was negative with respect to induction of aneuploidy and SCE.

Table 4.1.2.7.9 In vivo tests: Overview on rodent bone marrow tests with mice

Test system	Doses	Exp os. regi men	Sampl. times	Result	Local cytotox.	General toxicity	Remarks	Test substance	Reference
Micronu -cleus test	500 - 2000 mg/kg	2 x p.o.	48 h	negative	no	no	according to Guideline and GLP	Na ₃ NTA	BASF, 2004
Micronu -cleus test	200, 400 mg/kg (0.8-1.6 mmol/kg)	1 x i.p.	6, 12, 24 h	Equivo-cal	no	no data	screening procedure	Na ₃ NTA	Montaldi et al., 1988
Aneuplo idy test	138, 275 mg/kg (0.5-1.1 mmol/kg)	1 x i.p.	18 h	Negative	no	no data	screening procedure	Na ₃ NTA	Russo et al., 1989
SCE test	138, 275 mg/kg (0.5-1.1 mmol/kg)	1 x i.p.	18 h	Negative	no	no data	screening procedure	Na ₃ NTA	Russo et al., 1989

4.1.2.7.10 In vivo tests: Rodent germ cell tests

Rodent germ cell tests are available for trisodium nitrilotriacetic acid (Na₃NTA) and nitrilotriacetic acid (NTA).

Costa et al. (1988) reported that Na₃NTA induced aneuploidy in spermatocytes of C5BL/Cne x C3H/Cne F1 mice after single intraperitoneal exposure to 275 mg/kg (1.1 mmol/kg), exposure to 138 mg/kg was negative. Spermatocytes in metaphase II were sampled 6 h after treatment (and 3 h after colchicine exposure). After analysis of 6 animals (100 cells per animal) 3.5% hyperhaploidies were found in the 275 mg/kg group as compared to 0.5% in the control group. The historical negative control value was 1.2% (26 mice, 2243 spermatocytes); a positive control group was not employed. Data on toxicity were not given; in pre-tests the tested doses were in the 'high toxicity level'.

In a later publication from the same group, using the same methodology, again increased hyperhaploidy frequencies were found in mouse spermatocytes after single intraperitoneal administration of 275 mg/kg Na₃NTA (Zordan et al., 1990). In this investigation, in 3 males 3.3% hyperhaploid spermatocytes were found (negative control: 0.4%); again no positive control group was included.

RCC-CCR (2000) performed a re-analysis of the investigation by Costa et al. (1988) with the modification that single oral treatments of 100, 330 and 1000 mg/kg were used in NMRI mice; it was demonstrated that 1000 mg/kg was the maximum tolerated dose. Treatment groups consisted of 8 to 9 animals; 100 spermatocytes II were analysed per animal; sampling was 6 h after dosing. Hyperhaploidy frequencies in the treated groups ranged from 0.13 to 0.5%; the negative control value was 0.5%. In the 7 animals of the positive control group, with i.p. treatment with 200 mg/kg diethylstilbestrol, the hyperhaploidy frequency was 1.0% (with a standard deviation of 1.15%) and the difference to the negative control was without statistical significance. The investigation suffers from obvious methodological problems, e.g. in the positive control group only 7 out of 12 mice were scorable. The authors discussed that the test system 'was never validated'.

In screening assay for induction of dominant lethal mutations in male mice NTA was negative after single intraperitoneal administration of 125 mg/kg (0.65 mmol/kg) or 5 daily administrations of 1000 mg/kg (5.23 mmol/kg) by gavage (Epstein et al., 1972). 10 males were used per group, each treated male was caged with 3 untreated virgin females which were replaced weekly for 8 consecutive weeks. No detailed results were given.

Summary of rodent germ cell tests: No fully reliable germ cell tests are available. There is limited evidence that i.p. dosing of 275 mg/kg has the potential for induction of aneuploidy in mouse spermatocytes. However, the validity of the test system and the reliability of data are questionable. In a screening dominant lethal assay NTA was negative.

Table 4.1.2.7.10 In vivo tests: overview on rodent germ cell tests with mice

Test system	Doses	Exposure regimen	Result	General toxicity	Test substance	Remarks	Reference
Aneuploidy, spermatocytes II	138, 275 mg/kg (0.5-1.1 mmol/kg)	1 x i.p.	positive	'high toxic level'	Na ₃ NTA	no positive control, sampling time 6 h	Costa et al., 1988
Aneuploidy, spermatocytes II	275 mg/kg (1.1 mmol/kg)	1 x i.p.	positive	no data	Na ₃ NTA	no positive control, sampling time 6 h	Zordan et al., 1990
Aneuploidy, spermatocytes	100, 330, 1000	1 x p.o.	Negativ	MTD	Na ₃ NTA	positive control	RCC-CCR,

II	mg/kg		e			was negative	2000
Dominant lethal assay	125 mg/kg (0.7 mmol/kg)	1 x i.p.	Negative	no data	NTA	screening procedure	Epstein et al., 1972
Dominant lethal assay	1000 mg/kg (5.2 mmol/kg)	5 x p.o.	Negative	no data	NTA	screening procedure	Epstein et al., 1972

4.1.2.7.11 In vivo tests: Tests with *Drosophila melanogaster*

Drosophila tests are available for trisodium nitrilotriacetic acid (Na₃NTA) and nitrilotriacetic acid (NTA).

In *Drosophila* tests Na₃NTA or NTA were analysed with respect to various genetic endpoints. In all investigations, test compounds were administered via food (up to 50 mmol/l in substrate); Kramers (1976), Woodruff et al. (1985) and Mason et al. (1992) also used injections (up to 10 mmol/l in injection fluid).

In the sex-linked recessive lethal mutations assay (SLRL, Basc, Muller-5) in which mainly gene and small chromosomal mutations can be detected, negative results were obtained with Na₃NTA by Costa et al. (1988b) or with NTA by Kramers (1976), Woodruff et al. (1985), and Mason et al. (1992). With injection of NTA Woodruff et al. (1985) reported on an inconclusive finding.

Two investigations on germ cell aneuploidy were positive for chromosome loss (Ramel and Magnusson, 1979) or for chromosome gain (Costa et al., 1988a). Also in the SMART assay (somatic mutation and recombination test) positive results were obtained (Zordan et al., 1990; 1991). The authors conclude that Na₃NTA was positive for recombination and possibly for aneuploidy.

Summary for *Drosophila* tests: In *Drosophila* tests Na₃NTA or NTA were negative for recessive and dominant lethal mutations but positive for aneuploidy and recombination.

Table 4.1.2.7.11 In vivo tests: Overview on tests with *Drosophila melanogaster*

Test system	Doses	Exposure period	Result	Test substance	Remarks	Reference
Germ cell genotoxicity	50 mmol/l in substrate	3 days	negative	NTA	SLRL and dominant mutation test	Kramers, 1976
Germ cell genotoxicity	injection of 10 mmol/l	single administrat.	negative	NTA	SLRL and dominant mutation test	Kramers, 1976
Germ cell genotoxicity	up to 4000 ppm in	3 days	inconclusive	NTA	SLRL test	Woodruff et al., 1985

Test system	Doses	Exposure period	Result	Test substance	Remarks	Reference
	substrate					
Germ cell genotoxicity	injection of 1100 ppm	single administr.	negative	NTA	SLRL test	Woodruff et al., 1985
Germ cell genotoxicity	50 mmol/l in substrate	not given	negative	Na ₃ NTA	SLRL test	Costa et al., 1988b (MR 204); Gava et al., 1989
Germ cell genotoxicity	4000 ppm in substrate	3 days	negative	NTA	SLRL test	Mason et al., 1992
Germ cell genotoxicity	injection of 1100 ppm	single administr.	negative	NTA	SLRL test	Mason et al., 1992
Germ cell aneuploidy	4000 ppm in substrate	larvae period	positive	'NTA'	screening for X chrom. loss or gain	Ramel and Magnusson, 1979
Germ cell aneuploidy	50 mmol/l in substrate	3 days	positive	Na ₃ NTA	FIX test system	Costa et al., 1988a (EMM 12)
Somatic cell genotoxicity	50 mmol/l in substrate	24 h	positive	Na ₃ NTA	SMART test system	Zordan et al., 1990
Somatic cell genotoxicity	5 - 50 mmol/l in substrate	42 h	positive	Na ₃ NTA	SMART test system	Zordan et al., 1991

4.1.2.7.12 In vivo tests: Local genotoxicity tests in rat kidneys

Trisodium nitrilotriacetic acid monohydrate (Na₃NTA.H₂O) and disodium nitrilotriacetic acid (Na₂NTA) were analysed for local genotoxicity in rat kidney cells with DNA base damage as genetic endpoint. In these studies the primary goal was to investigate Fe³⁺NTA effects, sodium salts of NTA were used for comparison. Furthermore, nitrilotriacetic acid (NTA) was investigated with respect to the induction of micronuclei and DNA strand breaks.

Toyokuni et al. (1994) analysed oxidative base damage to DNA of rat kidney cells after intraperitoneal treatment with Na₂NTA. A single intraperitoneal administration was given with a dose of 87 mg/kg (0.37 mmol/kg; with analyses 3 and 24 h after treatment), treatment for 19 days was conducted with doses ranging from 29 to 58 mg/kg. Under all experimental conditions, no increase was found for 10 types of DNA base modification, including 8-hydroxy-desoxyguanine (8-OH-dG), which were analysed quantitatively by GCMS (gas chromatography / mass spectrometry). Also Umemura et al. (1990a) reported that single intraperitoneal administration of 87 mg/kg Na₂NTA had no effect on the 8-OH-dG level in DNA of rat kidney cells.

Recently BASF (1998; see also Bahnemann et al., 1998) compared nephrotoxic effects of Na₃NTA.H₂O and Fe³⁺NTA (see 4.1.2.6). Groups of 5 male rats were fed with Na₃NTA.H₂O in doses of appr. 9 mg/kg (150 ppm in the diet) or 926 mg/kg (20000 ppm) for 4 weeks. 8-OH-dG levels (measured by HPLC) were not increased after treatment (0.65 8-OH-dG per 10⁵ dG in the negative control group, 0.01 and 0.84 in the treated groups). In the 20000 ppm-group, BrdUrd labelling was used to determine frequencies of S-phase cells in the cortex, the outer stripe of the outer medulla and the inner stripe of the outer medulla. No effect on cell replication was found in the cortex region after exposure for 1 week, whereas 10- to 19-fold increases in S-phase cells were observed after 4-week exposure.

A new protocol for investigation of micronuclei induction in rat kidney was used by Robbiano et al. (1999). For stimulation of cell proliferation (which is needed for micronucleus formation) rats were subjected to unilateral nephrectomy and, 24 h later, to i.v. injection of 250 mg/kg folic acid. Three animals per group were dosed orally 1x with 735 mg/kg or 3x on 3 consecutive days with 490 mg/kg; these doses correspond to ½ and 1/3 of the LD₅₀. Increased micronuclei frequencies of 0.14% and 0.27% were found; the latter frequency was statistically different from a historic negative control of 0.07%. Due to encumbering and non-validated protocol, the lack of a concurrent negative control and the use of very high doses the biological significance of these findings is interpreted as being inconclusive.

In the same animals that were analysed for micronuclei frequencies Robbiano et al. (1999) also investigated DNA strand breaks in kidney cells by use of an alkaline version (pH 13) of the comet assay. Increases in tail length from 3.2 µm in the (historic) negative control to 4.9 µm (1x 735 mg/kg) and 10.8 (3x 490 mg/kg) were found. For analysis of DNA strand breaks the same encumbering test protocol was used as for micronucleus analysis, although unilateral nephrectomy and treatment with folic acid are not needed in the comet assay (the ability for investigating genotoxicity in non-proliferating tissues is the main advantage of this test system.). Furthermore, with 3.2 µm the tail length in the negative control is astonishing low. Altogether, the comet assay findings are evaluated as being inconclusive.

Summary for local genotoxicity tests in rat kidneys: Sodium salts of NTA did not induce DNA base damage in rat kidney cells after oral exposure to doses up to 926 mg/kg for 4 weeks or after intraperitoneal exposure for doses up to 87 mg/kg (0.37 mmol/kg). Inconclusive findings were obtained for NTA effects on induction of micronuclei and DNA strand breaks.

Table 4.1.2.7.12 In vivo tests: Overview on local genotoxicity tests in rat kidney cells

Genetic end-point	Doses	Expos. regimen	Sampl. times	Result	General toxicity	Test substance	Remarks	Reference
DNA base damage	87 mg/kg (0.37 mmol/kg)	1 x i.p.	1-24 h	negative	no data	Na ₂ NTA	8-OH-dG as genetic endpoint	Umemura et al., 1990a
DNA base damage	87 mg/kg (0.37 mmol/kg)	1 x i.p.	3-24 h	negative	no data	Na ₂ NTA	8-OH-dG and 9 further DNA modifications as genetic endpoint	Toyokuni et al., 1994

Genetic end-point	Doses	Expos. regimen	Sampl. times	Result	General toxicity	Test substance	Remarks	Reference
DNA base damage	29-58 mg/kg (0.12-0.25 mmol/kg)	i.p., 19 days		negative	no data	Na ₂ NTA	8-OH-dG and 9 further DNA modifications as genetic endpoint	Toyokuni et al., 1994
DNA base damage	9 and 926 mg/kg	oral feed	4 weeks	negative	kidney toxicity	Na ₃ NTA.H ₂ O	8-OH-dG as genetic endpoint	BASF, 1998; Bahnemann et al., 1998
Micro-nuclei	- 490 mg/kg - 735 mg/kg	- 3x p.o. - 1x p.o.	1 - 2 days after last treatment	inconclusive	doses correspond to 1/3 - 1/2 of LD-50	NTA	unilateral nephro-ectomy, 24 h later i.v. -injection of 250 mg/kg folic acid (for stimulation of cell proliferation); lack of positive control	Robbiano et al., 1999
DNA strand breaks	- 490 mg/kg - 735 mg/kg	- 3x p.o. - 1x p.o.	1 - 2 days after last treatment	inconclusive	doses correspond to 1/3 - 1/2 of LD-50	NTA	unilateral nephro-ectomy, 24 h later i.v. -injection of 250 mg/kg folic acid; lack of positive control	Robbiano et al., 1999

4.1.2.7.13 Summary of mutagenicity data and conclusion

There are a number of genotoxicity tests with sodium salts of NTA; however, the majority of data is of relatively low reliability.

In bacteria sodium salts of NTA are negative for gene mutations. In mammalian cell cultures sodium salts of NTA did not induce gene mutations, sister-chromatid exchanges (SCE), DNA excision-repair (UDS) or DNA strand breaks. NTA seems to have a weak potential for induction of chromosomal aberrations / micronuclei; cell transformation assays were without clear result.

In vivo in mammals, sodium salts of NTA did not induce genetic effects in bone marrow cells - neither micronuclei and aneuploidy nor SCE. In kidney cells, results were negative for DNA base damage and inconclusive for induction of micronuclei and DNA strand breaks.

On the other hand, from in vivo germ cell tests there is limited evidence that i.p. dosing of 275 mg/kg has the potential for induction of aneuploidy in mouse spermatocytes. However, positive effects are limited to high doses and a non-linear dose-effect relationship, including a threshold, is to be assumed. Furthermore, these findings suffer from methodological insufficiencies and screening assays for dominant lethal mutations were negative.

Considering the amount of in vivo data with rodents, the results obtained with *Drosophila* do not add relevant information.

From the whole amount of data on mammalian somatic cells in vitro and in vivo, there is no plausible evidence for in vivo mutagenicity of NTA and its sodium salts. Therefore, the substances should not be classified as mutagens.

4.1.2.8 Carcinogenicity

No human data available.

4.1.2.8.1 Animal data

Studies with administration of NTA in its forms as $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$, and H_3NTA were considered as relevant information because these compounds dissociate into the nitrilotriacetate anion and the respective cations under physiological conditions. Data from studies with the following soluble, but strongly associated complexes of CaNaNTA , MgNaNTA , AINTA , ZnNaNTA or FeNTA were discarded from this section of the report.

Using the rat and the mouse as test species there are a number of oral studies with administration of NTA via the diet or the drinking water. Conventional cancer bioassays were conducted in rats (6 diet studies and 2 drinking water studies) and mice (1 diet study). Six studies using an initiation-promotion model were available for the rat and one study testing an initiation-promotion model was available for the mouse. A mouse lung tumor cancer study was also conducted. For the dermal route a single injection study in the mouse is available. The studies were reported in the order of the administration routes (inhalative, oral (feeding < drinking water studies, rat < mouse studies), dermal).

Animal studies with inhalative exposure:

No data available.

Animal studies with oral administration:

For overview, summaries on tumor incidences were given in Table 4.1.2.8.2A and B.

Feeding studies:

Comment on the doses in oral feeding studies: If not otherwise mentioned, calculated intake of the test substance is based on an average food intake of 7% of the bw/d for rats and of 10% of bw/d for mice. If no data on the purity of the test substance are reported, 100% purity is assumed.

RAT

Conventional carcinogenicity studies:

- In the NCI report (NCI, 1977), two rat cancer bioassays for the carcinogenicity of $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ were conducted in male and female F344 rats with ad libitum consumption

of food and water. Animals were observed daily for deaths and clinical signs and weighed every 2 weeks for the first 12 weeks and every 4 weeks later on. Gross and microscopic examination included all major tissues and organs (32 sites including kidney and bladder, but ureter was not routinely checked microscopically), or gross lesions. In the first study, Na₃NTA.H₂O was tested in groups of 24 males and 24 females at diet concentrations of 0, 200, 2000, and 20000 ppm (0, 0.7, 7, and 70 µmol/g diet, appr. 0, 9, 92, 921 mg NTA/kg bw/d calculation based on the molecular mass of 191 for NTA) for a 24-months period.

High dose males and females gained significantly less body weight (-12%, -10%, resp.). The mean survival time for the males was 92 weeks compared with 104 weeks in the other groups, the survival rate was significantly lower in the high dose males. Although 75% of the males died before termination of the study, at week 90 50% of the males were still alive. Primary neoplasms of the urinary tract were seen in 14/24 high dose males (carrying 9 kidney tumors, 8 ureter carcinomas and 1 urinary bladder tumor, see Table 4.1.2.8.1), 11 of which died before 104 weeks. Similar neoplasms were also observed in 13/24 high dose females (developing 4 tubular cell tumors, 6 ureter carcinomas and 5 urinary bladder carcinomas), 4 of which were among the 7 females that died before 104 weeks. The tumors consisted of pelvic transitional-cell carcinomas (males only), tubular-cell (adeno-) carcinomas, and tubular-cell adenomas of the kidney, transitional-cell carcinomas of the ureter and transitional-cell carcinomas of the urinary bladder (total no. of tumors in the urinary tract in Table 4.1.2.8.1 and in Table 4.1.2.8.2.A). A papilloma of the bladder was also present in a mid dose female. Transitional-cell carcinomas were metastatic in 5 males and 5 females of the high dose groups. Other tumor incidences were not associated to the NTA treatment.

Whereas hydronephrosis was evident only at the high dose level in most of the males and females, other lesions (hyperplasias, dysplasias) considered as presumable preneoplasias were registered at all three dose levels. Hyperplasia of tubular cells were observed in 20/24 males and 11/24 females of the high dose group. Increased numbers of transitional cell hyperplasia were seen in the renal pelvis, the ureter and in the urinary bladder of males and females at all dose groups (see Table 4.1.2.8.1). The increase was marked at the mid dose level where over half the females developed hyperplasias in the urinary bladder. This effect seems to be the most sensitive effect of life-time NTA treatment. A marginal (probably non-significant) increase of hyperplastic lesions at the low dose supports that this dose represents the non-effect level for preneoplastic lesions. In addition, epithelial dysplasia of urinary bladder epithelium was evident in 1 low dose and 4 mid dose males and in 1, 3, and 8 females of the low, mid, and high dose level (no one in controls). Dysplasia was also found in the ureter of 2/24 females in the high dose group.

Table 4.1.2.8.1 Summary on incidences of hyperplasias, dysplasias and tumors of the kidney tubular cells and of the transitional cell epithelium (TC) in the urinary tract of rats (NCI, 1977)

Dosis (% test substance in diet)	mg/kg	Control group	9 mg/kg/d (0.02%)	92 mg/kg/d (0.2%)	921 mg/kg/d (2%)

No. of animals/ sex examined:	24 m	24 f	23 m	24 f	24 m	24 f	24 m	24 f
Tubular cell Hyperplasia Adenoma Adenocarcinoma							20 3* 1	11 3 1
Renal pelvis TC hyperplasia TC carcinoma	1	1		2	3		7 4	10
Ureter TC hyperplasia TC dysplasia TC carcinoma			1		4		3 β 8	5 2 6
Urinary bladder TC hyperplasia TC dysplasia TC papilloma TC carcinoma			3 1	1 1	3 4	13 3 1	8 1	14 8 5

* plus 1 haemangioma

Inflammatory processes of the kidneys were found in most of the control, low, and mid dose male, but not in females and high dose males.

Considering the hyperplastic and dysplastic lesions observed in all dose levels as presumable preneoplastic lesion, the NOAEL for (noncancer) toxic lesions was estimated to be 2000 ppm (appr. 92 mg/kg bw/d). This is based on the findings of mortality increase and renal hydronephrosis at the high dose level.

A reevaluation of renal nonneoplastic lesions of a part of the high dose animals (24) of this study (Alden & Kanerva, 1982) resulted a figure demonstrating that age-related nephrosis and basophilic/eosinophilic hyperplasia occurred in all treated animals compared to half of 10 control animals reevaluated. The mean severity of nephrosis was increased in high dose animals versus in the control group. Tubular vacuolation and hyperplasia of the vacuolated cell type was seen in all high dose animals reexamined versus in none of the controls. In addition nodular hyperplasia of the vacuolated cell type and of the basophilic/eosinophilic cell type was found more than half of the treated animals. Adenomatous hyperplasia was seen in 17% of reexamined animals of the high dose group but not in the control group. As this reevaluation is limited to this spare data and single dose without any further information about grading, sexes or selection criteria on animal examined, original NCI data were considered of higher relevance for this report.

- In the second study of the NCI report (1977), F344 rats were exposed to diet concentrations of a) 0, 7500, and 15000 ppm Na₃NTA (0, 40, and 80 μmol NTA/kg feed, appr. 0, 526, and 1053 mg NTA/kg bw/d), or b) the same diet concentrations of Na₃NTA.H₂O (0, 27, and 55 μmol NTA /kg feed, appr. 0, 355, 724 mg NTA/kg bw/d) for a period of 18 months followed by a treatment-free period of 6 months. Each study included 50 males and 50 females in the treatment groups and 20 males and 20 females in the control groups. Test parameters were similar to the above mentioned for the 24 months study except that weighing was performed weekly during the first 6 weeks, biweekly for the next 6 weeks, and monthly thereafter.

a) In the NTA groups, survival was similar in treated and control groups of both sexes, the average weight was reduced in a dose-related manner in both dose groups of each sex. Tumors of the urinary system considered to be related to the treatment were found in 7 high dose males, 1 low dose male, 14 high dose females and 2 low dose females ((numbers of tumors see Table 4.1.2.8.A) These tumors were tubular-cell adenoma, tubular-cell adenocarcinoma, pelvic transitional cell papilloma of the kidneys, transitional cell papilloma and papillary adenoma of the ureter, transitional-cell carcinoma and squamous cell carcinoma of the bladder. Livers of 8 of the low dose females and of 22 of the high dose females had neoplastic nodules (syn. to hepatocellular adenoma) compared to 2 in the controls. Hepatocellular carcinomas occurred in 3 low dose males, and neoplastic nodules appeared in 3 controls, 2 low dose, and 2 high dose males. Slightly higher incidences of alveolar/bronchiolar adenomas and carcinomas and pheochromocytomas in treatment groups than in control groups were observed. Nonneoplastic changes associated to NTA consisted of higher incidences of chronic inflammation in the kidneys. Hyperplastic lesions of the urinary system were only seen in the treated rats. Transitional epithelial hyperplasia of the urinary bladder was present in 2 low dose males, 2 low dose females, 1 high dose male, and 11 high dose females. A similar lesion was seen in the ureter of 1 high dose male. A single male of the low and high dose had a renal (cell) hyperplasia. The LOAEL for (noncancer) toxic lesions (hyperplasias were not considered) was estimated to be 7500 ppm (appr. 526 mg/kg bw/d).

b) The Na₃NTA.H₂O study revealed a dose-related reduction of body weights in rats of both sex. Survival rates of all groups did not show significant differences compared to the controls. Higher numbers of tumors of the urinary system were observed in both dose groups, no such tumor occurred in the controls (see Table 4.1.2.8.A). One low dose male each developed a tubular-cell adenoma of the kidney, respectively a papilloma of the ureter. In the high dose males a tubular-cell carcinoma of the kidney was found in one animal and a papilloma of the ureter in another. Urinary tract tumors in female rats were confined to the bladder consisting of one transitional cell carcinoma and a papilloma in the high dose group, and a squamous cell carcinoma and in three additional females transitional cell carcinomas in the low dose group. Increased incidences of chronic inflammatory changes were evident in both dose groups of both sexes versus the control groups. Severity was reported to be less severe in controls. Toxic nephropathy was found only in 2 high dose females. Epithelial hyperplasia of transitional cells was not seen in controls but in the renal pelvis of 3 high dose males, the ureter of 1 high dose male, and the urinary bladder of 3 high dose males, 4 low dose females and 5 high dose females. A single male and female of the high dose level presented a tubular cell hyperplasia in the kidneys.

With respect to the nonneoplastic findings (except the hyperplastic lesions), the LOAEL of Na₃NTA.H₂O of this study were diet concentrations of 7500 ppm (equiv. 355 mg NTA /kg bw/d).

Initiation-promotion studies

The results of NTA-treated animals without any initiation treatment were included in the Table 4.1.2.8.A

- There were three initiation-promotion studies on the development of tumors of the urinary bladder. In one of them (Kitahori et al., 1988), promoting effects of NTA was tested in groups of 15-20 male Wistar rats after a 2-week initiation treatment with 0.2% N-bis(2-hydroxypropyl)-nitrosamine (DHPN) in the drinking water. Thereafter, animals were fed with diet containing 1% Na₃NTA.H₂O, 1% Na₃NTA.H₂O plus 1% NH₄Cl, 1% H₃NTA, 1% H₃NTA plus 1% NH₄Cl, or without these chemicals (initiation-only) for 28 weeks. Other groups received the same NTA-formulations without initiation and one control group remained untreated. Decreases in urinary sodium ion concentration and urinary pH were expected in groups that received cotreatment with NH₄Cl. The urinary bladders were inflated with formalin for fixation, weighed and processed for histopathological examination. At the end of experiment, relative urinary weights did not differ between animal groups. No consistent finding on the urinary volume and the urinary pH values was found in the NTA-treated groups. Elevated numbers of rats with hyperplastic lesions (simple, papillary, nodular hyperplasia) in the urinary bladder was observed in all groups initiated with DHPN and treated with any of the NTA-containing diets. Tumors of the urinary bladder occurred in the initiation-only group (1 papilloma) and in both Na₃NTA.H₂O groups (6 papillomas and 3 transitional cell carcinomas in the group without NH₄Cl, 1 papilloma in the group with NH₄Cl), and 1 papilloma in the group with H₃NTA without NH₄Cl. The number of animals bearing hyperplasias or tumors of the urinary bladder was fewer in the group with NH₄Cl cotreatment, resulting to the conclusion that NH₄Cl reduced the tumor response. Moreover, hyperplasias of the urinary bladder developed in all NTA-groups without the initiation. Na₃NTA.H₂O containing feed induced simple hyperplasias in 11 of 15 rats, but not in untreated controls. 1/15 animals with simple hyperplasias were seen in each of the other Na₃NTA.H₂O and H₃NTA groups (with/without NH₄Cl).
- In second initiation-promotion study of this working group (Kitahori et al., 1985), 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), known to induce tumors of the urinary bladder and the kidneys, was administered in drinking water for 4 weeks to groups of 21 male Wistar rats. Thereafter, Na₃NTA.H₂O at concentrations of 0.3%, 0.5%, and 1% was fed with the diet for 28 weeks. Additional groups received the Na₃NTA.H₂O containing diets without initiation and one single group were treated with BBN without promotion treatment (no control group). Animals of all seven groups were killed in week 32. The mean body weights were significantly reduced in dose groups receiving the high dosage of NTA with or without initiation treatment. The urinary bladder and the kidneys were weighed. The urinary bladders were inflated with formalin for fixation, and processed for histopathological examination. The kidney weights were higher in most of the Na₃NTA.H₂O groups compared to the initiation-only group, no consistent weight change was seen in the urinary bladder. Dose-related high incidences of simple hyperplasia of the urinary bladder were found in all Na₃NTA.H₂O groups with (85, 100, 100 %) and without initiation (61, 80, 100 %) compared to 25% of the rats after initiation-only treatment. Papillary/nodular hyperplasias of the urinary bladder were also evident in all dose levels of the rats

treated with BBN and Na₃NTA.H₂O (61, 100, 100 %) compared to 15% of animals of the initiation-only group. There was a dose-related increased number of papillomas (6, 44, 70%) and transitional cell carcinomas (4, 11, 40%) of the urinary bladder in rats receiving initiation and promotion treatment, but no tumors in the groups without initiation or the initiation-only group.

- The BBN was also administered in another initiation-promotion model (Hiasa et al., 1985a) to elucidate the promoting activity of Na₃NTA.H₂O. Groups of 19-21 male Wistar rats were given drinking water containing 500 ppm BBN for 4 weeks and then put on diet containing 10000 ppm Na₃NTA.H₂O for 28 weeks or were fed with 10000 ppm Na₃NTA.H₂O without initiation treatment. Additional groups exposed to the initiation treatment only or remained untreated served as controls. Histopathological examination was limited to the urinary bladder. Mean body weights and kidney weights were lower in the Na₃NTA.H₂O treated groups (with or without initiation) than in the groups without Na₃NTA.H₂O. An increased number of simple hyperplasias of the urinary bladder was observed in the all treatment groups (Na₃NTA.H₂O only 37% < BBN only 61% < BBN plus Na₃NTA.H₂O 100%), but not in the untreated control group. No other change of the urinary bladder was seen in the Na₃NTA.H₂O only group. Whereas the BBN-only group also developed papillary/nodular hyperplasias of the urinary bladder (61%), BBN plus Na₃NTA.H₂O resulted in papillary/nodular hyperplasias (100%), papillomas (90%), transitional cell carcinomas (25%) and squamous cell carcinoma (5%) of the urinary bladder.
- In contrast to these studies, a 4-week administration with 0.01% and 0.05% BBN with the drinking water with or without a subsequent feeding for 32 weeks of diet containing 2% (20000 ppm) of Na₃NTA.H₂O to groups of 25-26 F344 rats did not clearly demonstrate a promoting effect of Na₃NTA.H₂O (Fukushima et al., 1985).

All groups treated with NTA gained less weight than those with BBN only (data not shown). The number of simple hyperplasias, papillary/nodular hyperplasia and rats bearing papillomas and carcinomas of the urinary bladder were increased in the 0.05% BBN plus Na₃NTA.H₂O-group (incidences 92%, 89%, 69%, 19%) versus the 0.05% BBN-only group (77%, 50%, 35%, 4 %). However, at 0.01% BBN, a higher number of animals with simple hyperplasias (80%), and papillomas (44%) and carcinomas (4%) of the urinary bladder occurred in the 0.01% BBN-only group in comparison to the group receiving 0.01% BBN plus Na₃NTA.H₂O (incidences 79%, 38%, 0%, resp.). Hyperplasias of the urinary bladder epithelium or other effects were not seen in the Na₃NTA.H₂O-only group without initiation treatment.

- In a fifth initiation-promotion tumor model using a nitrosamine as an initiating agent, the influence of Na₃NTA.H₂O on the development of kidney tumors was investigated (Hiasa et al., 1985b). Four groups of 24 male Wistar rats were fed with diet containing 1000 ppm N-ethyl-N-hydroxyl nitrosamine (NEELA) for two weeks. Thereafter this groups received 0, 3000, 10000, or 30000 ppm Na₃NTA.H₂O with the diet for 30 weeks. Three additional groups were fed with Na₃NTA.H₂O containing diet with the same concentrations without initiation, another group served as untreated control. 4/24 animals (16%) of the initiation-only group developed kidney tumors (tumor types not specified), simple and adenomatous hyperplasias were also seen. A similar incidence of renal tumors (5/22, 22%) were found in the group with initiation + 3000 ppm Na₃NTA.H₂O treatment. 100% of animals of the initiation + 10000 ppm or 30000 ppm Na₃NTA.H₂O treatment had tumors of the kidneys. Related to the Na₃NTA.H₂O doses, the number of tumors/animal increased in the initiated groups. None of the

treatment groups receiving Na₃NTA.H₂O without initiation developed renal tumors. A dose-related increase of simple hyperplasias (mean no. of lesions/cm²) in the low and mid dose groups of Na₃NTA.H₂O with or without initiation was seen. Additionally, adenomatous hyperplasias were evident in all initiated groups which were also treated with Na₃NTA.H₂O. At 30000 ppm Na₃NTA.H₂O (with or without initiation), a diffuse tubular hyperplasia in between cystic lesions was observed. Severe graded cystic lesions and hydronephrosis were seen in both high dose groups of Na₃NTA.H₂O irrespective of the initiation treatment.

- The sixth initiation-promotion study (Hiasa et al., 1984) also focussed on the effects on the kidneys used 1000 ppm N-Ethyl-N-hydroxyethylnitrosamine in the diet for a 2-week initiation treatment of groups of 24 male Wistar rats. Subsequently, the rats were given a diet containing 500 ppm or 10000 ppm of Na₃NTA.H₂O for 30 weeks. The study was terminated during week 32. Two additional groups received Na₃NTA.H₂O of the same concentrations only (without initiation). One initiation-only group and an untreated group served as controls groups. Early deaths occurring at all three initiated groups (3 rats in the 10000 ppm group, 1 rat in the 500 ppm group, 3 rats in the initiation-only group) were excluded from the investigations on the kidney effects. Significantly lower body weights were observed in both Na₃NTA.H₂O groups (with and without initiation) and in the 500 ppm Na₃NTA.H₂O group plus initiation. Mean kidney weights was not significantly changed between the groups. Renal tumors were observed in all initiated groups. Most of the renal tumors seen were located in the cortex, a few of them were found in the medulla, and no one was observed in the renal pelvis. The number of rats with renal tumors was 21 in the 10000 ppm group, 9 in the 500 ppm group, and 7 in the initiation-only group. None of the animals treated with Na₃NTA.H₂O or left untreated developed tumors of the kidneys. The mean numbers of atypical tubular cell foci/cm² were increased in all initiated groups (3.7 in the initiation-only group, 7.3 in the 500 ppm group, 17 in the 10000 ppm group). Atypical cell foci (1/cm²) was also found in the 10000 ppm Na₃NTA.H₂O group without initiation, but did not occur in the 500 ppm group without initiation and the untreated controls. Slight cystic dilatation of the tubules was seen in few rats in all initiated groups. No tubular casts or interstitial fibrosis was seen in any rat. The renal pelvis presented a dose-related hyperplasia of the transitional epithelium in the 500 ppm and 10000 ppm Na₃NTA.H₂O groups (with and without initiation).

MOUSE

Conventional carcinogenicity studies

- The third study of the NCI report (1977) was on groups of B6C3F1 mice receiving a diet containing 0, 7500 ppm, and 15000 ppm of NTA (0, 40, and 80 µmol NTA/kg feed, appr. 0, 752, and 1504 mg NTA/kg bw/d, calculation based on molar weight of NTA of 191) and 0, 2500 ppm, and 5000 ppm of Na₃NTA.H₂O (0, 9, and 18 µmol NTA/kg feed, appr. 0, 169, and 338 mg NTA/kg bw/d) for a period of 18 months and a treatment free period of 3 months. Each treatment group contained 50 males and 50 females, 20 males and 20 females served as controls. Testing procedures were similar as described above for the F344 rat groups of the NCI report fed with NTA or Na₃NTA.H₂O.

a) The NTA treatment resulted in lower body weights of the high dose males and of females of the mid and high dose groups when compared with those of the controls. The survival rates of all experimental groups were comparable. Similar to the rat, treatment-related tumors were evident in the urinary system in a total of 32 male and female mice (no. of tumors see Table 4.1.2.8.A). Tubular cell adenocarcinomas, were found in 22 high dose males, 4 high dose females and in 5 low dose males, five of these tumor-bearing high dose animals had bilateral adenocarcinomas. A tubular cell adenoma was observed in another high dose male. A papilloma of the renal pelvis occurred in one high dose male. No renal neoplasms were present in controls or low dose females. Nonneoplastic findings such as hydronephrosis were found in 8 high dose males, 3 low dose males and 12 high dose females, but no lesions were seen in controls or low dose females. Except that tubular degeneration was evident in 4 high dose females and one high dose male and inflammation of the kidney in 5 high dose males, other nonneoplastic findings were observed in few animals without being related to the NTA treatment. Hyperplastic lesions were found in 1 low dose male (transitional epithelial hyperplasia of the ureter) and in 1 high dose female (transitional epithelial hyperplasia of the renal pelvis). The LOAEL for nonneoplastic findings (except hyperplasias) were diet concentrations of 7500 ppm for NTA (752 mg/kg bw/d).

b) Na₃NTA.H₂O treatment also reduced average weights of males and females in a dose-related manner. Survival rates did not show differences between all groups of both sexes. No tumors of the urinary system were observed. No other tumor could be clearly related to the treatment.

Hydronephrosis was the only treatment-related lesion of nonneoplastic nature occurring in 1 low dose males, 28 high dose males, 1 low dose female, and 30 high dose females.

The LOAEL for nonneoplastic findings were diet concentrations 2500 ppm for Na₃NTA.H₂O (169 mg NTA /kg bw/d).

Initiation-promotion studies

The promoting effect of NTA on the tumor development in the urinary bladder and the kidneys was also confirmed in a mouse study (Matsuki et al., 1992). Groups of 14-16 male C3H/He mice were given 0.05% BBN with the drinking water for 8 weeks. Thereafter, 1% Na₃NTA.H₂O or 1% H₃NTA (appr. 1000 mg/kg bw/d) were administered with the diet for 12 weeks until the end of the study at week 20. Groups treated with Na₃NTA.H₂O and H₃NTA containing diets after initiation presented a higher percentage of animals with dysplasias (33%, resp. 50% in NTA groups) and transitional cell carcinomas (TCC in 20% and 29%, resp.) of the urinary bladder than the initiation-only group (only TCC in 11%). No lesion was found in the urinary bladder of 10 untreated controls or in two further groups of 9-10 mice, which received 1% Na₃NTA.H₂O or 1% H₃NTA only from week 9 to week 20. Similarly, hyperplasia (20% and 21%) and transitional cell carcinomas (13% and 14%) of the kidney were evident in both BBN-NTA (Na₃NTA.H₂O and H₃NTA) groups, but not in the initiation-only group (BBN-alone). 1/10 mice of the H₃NTA group also demonstrated a hyperplastic lesion of the kidney, whereas no kidney lesion was found in the Na₃NTA.H₂O group or untreated controls.

Drinking water studies:

Comment on the doses in oral drinking water studies: If not otherwise mentioned, calculated intake of the test substance is based on an average water intake of 10% of the bw/d. If no data on the purity of the test substance is reported, 100% purity is assumed.

RAT

Conventional carcinogenicity studies:

- Two groups of 204 and 184 non-inbred Sprague-Dawley male rats (average body weights of 70 g, resp. 350 g) were divided each in one control group (group I and IV) and one treatment group (group II and group III) which received deionized water ad libitum containing 0.1% Na₃NTA (appr. 100 mg/kg bw/d) (Goyer et al., 1981). All animals surviving 704 days beyond the start of the experiment were killed. Tissues routinely taken for histological examination included thyroid gland, lungs, heart, liver, spleen, kidneys, adrenal gland, pancreas, brain, gonad, and skeletal muscle. Sections of bone, pituitary gland, ureter, urinary bladder, and other nonspecific tissues were obtained for histology only if an abnormality was detected by gross inspection, however the ureter and urinary bladder were not opened. In addition, eyes, bone and gastrointestinal tract were examined from 10 randomly selected control and 10 experimental animals in the absence of any gross abnormality.

No significant treatment-related effect was seen on body weight gain. Cumulative mortality curves did not differ significantly between the groups I and II, resp. III and IV. In the pooled groups, a significant higher rate of Na₃NTA-treated animals died before day 550 (19.7% vs. 11.2% in control groups).

While only 5 control rats (of 186) had renal (tubular-cell) adenomas, a statistically significant increase of renal tumors were seen in the Na₃NTA-treated groups. 25 rats with renal adenomas and 4 rats with renal (tubular-cell) adenocarcinomas were observed in a total of 183 rats of group II and III. No significant differences were observed for any of the other tumors between the Na₃NTA-treated rats and control groups and transitional cell neoplasias were absent.

The overall incidences of renal tubular cell hyperplasia and nephritis were similar in the treated and control groups reaching 85% of animals. However, a significantly greater number of Na₃NTA-treated rats had more severe grades of hyperplasia (44/186 controls vs. 67/183 treated animals). The findings suggested that Na₃NTA treatment was related to a higher incidence of severe hyperplasia and renal tumors, but not to nephritis.

Considering the severe graded hyperplasias as possible preneoplastic lesions, Na₃NTA did not cause toxic effects. Therefore a NOAEL for (nephro-)toxicity was 0.1% Na₃NTA (appr. 100 mg/kg bw/d).

- In a less reliable cancer study Lijinsky et al. (1973) were unable to confirm a carcinogenic potential of Na₂NTA. 0.5% Na₂NTA (appr. 400 mg/kg bw/d, based on an average weight of 250 g) without or with 0.2% sodium nitrite (NaNO₂) was given via drinking water to groups of 15 male and 15 female MRC rats for up to 84 weeks. The dose was 20 ml solution per animal on 5 days a week (100 ml/cage with 5 animals), which were consumed by morning. At week 104, surviving animals were killed and grossly observed tumors were confirmed by microscopic examination. No sign of toxic effects were observed, and most animals survived until the end of treatment. There was no significant difference in tumor incidence or organ distribution of tumors

in both Na₂NTA-treated groups compared to the controls. With respect to the urinary system, one kidney tumor was seen in the males receiving Na₂NTA, 2 kidney tumors were evident in males receiving Na₂NTA+NaNO₂. The number of tumor bearing animals was 9 males and 7 females in the Na₂NTA group and 8 males and 9 females in the Na₂NTA+NaNO₂ early group in comparison to 5 males and 4 females in the control group. The results were not in accordance to that of other cancer studies. However, the study included only few animals, clinical parameters, microscopic examination and study documentation were very limited compared to standard test protocols. No significant effect was contributed to the added NaNO₂ which was added to examine effects after the formation of N-nitroso compounds from NTA and nitrite.

MOUSE

Lung tumor cancer studies:

- In an early study of Greenblatt and Lijinsky (1974) the development of lung tumors were examined in Swiss mice orally treated with Na₂NTA for 26 weeks and killed at 35-36 weeks. Groups of 40 males and 40 females received 5 ml drinking water/mouse on 5 days/week containing 5 g Na₂NTA/l (appr. 167 mg/kg bw/d, based on an average weight of 30 g), or 2 g N-nitrosoiminodiacetic acid (NIDA)/l (results on this group omitted from this report) and controls remained untreated, or received 1 g/NaNO₂/l, or 0.1 g N-nitrosopiperidine (NP)/l as a positive control. The mice were weighed every 4 weeks and checked twice daily. A complete autopsy was performed and lung adenomas were confirmed by histological examination, which was also performed on 23 organs/tissues including the kidneys, urinary bladder and bone. Na₂NTA-treated groups did not show differences in weight, survival or evidence of gross toxicity. There was no significant increase in lung adenomas or other tumors in mice treated with Na₂NTA or Na₂NTA plus NaNO₂ when sexes were compared separately. If the number of lung adenomas was combined in the group receiving Na₂NTA plus NaNO₂, a significant higher incidence compared to controls was evident (22 adenomas vs. 11). In contrast to this, the group receiving pure Na₂NTA with drinking water had nonsignificantly lower rate of lung ademas than controls. Tumors of the urinary system were reported as 2/76 control animals of both sexes with a tubular adenoma of the renal cortex, resp. a (glom)angioma of the urinary bladder and 1 out of 74 animals with a tubular adenoma of the renal cortex in the Na₂NTA group. NP-treated mice showed a tenfold increase in lung adenomas/mouse compared to the controls. No renal or osseous changes were detected by light microscopy. Compared to standard testing protocols of cancer studies, the treatment duration was too short, and the number of animals was too low.

The NOAEL for toxic effects in this study was 20 ml solution containing 5 g Na₂NTA/l (appr. 167 mg/kg bw/d).

Animal studies with dermal administration:

MOUSE

- In an early study of Van Duuren et al. (1974), female ICR/Ha Swiss mice were injected subcutaneously with 3.5 mg NTA in 0.05 ml distilled water once a week for

up to 580 days. One of 30 animals treated had an adenocarcinoma of the mammary gland near to the injection site, none of the animals treated and of 100 control animals developed a skin tumor.

4.1.2.8.2 Summary on NTA carcinogenicity in animals

Table 4.1.2.8.2.A Summary of results from medium and long-term/cancer studies on rats: Urinary tract histomorphology

Substance	Administration route	Duration of treatment	Dose NTA % in DW or diet (mg/kg/d)	Urinary tract neoplasms ^a				Hyperplastic lesions	Nephrotoxicity	Reference
				Renal Tubular-cell neoplasms	Renal Transitional-cell epithelium neoplasms	Ureter Transitional-cell epithelium neoplasms	Bladder Transitional-cell epithelium neoplasms			
Na ₂ HNTA	DW	84 wk (20 ml DW/day, 5d/wk)	0% 0.5% 0.5%†	0/30 [§] 1/30 ^{§§} 2/30	-	nd	nd	nd	nd	Lijinsky, Greenblatt & Kommineni, 1973
Na ₃ NTA	DW	704 d	0% 0.1% (100 mg/kg/d)	5/186 29/183	-	-	0/186 0/183	0.1%: higher severity grades of renal hyperplasias	-	Goyer et al., 1981
Na ₃ NTA	Diet	24 mo (interim kill at 6, 12, 19 mo, 5 rats/sex/group)	0% 0.03% (9 mg/kg/d) 0.15% (97 mg/kg/d) 0.50% (322 mg/kg/d)	-	-	nd	-	-	≥0.15%, ≥6 mo: nephrosis: hydropic degeneration, dilation, hemosiderin, protein casts, interstitial inflammation of renal tubules	Nixon, Buehler & Niewenhuis, 1972
Na ₃ NTA	Diet	24 mo	0% 0.02% (9 mg/kg/d) 0.2% (92 mg/kg/d)	0/48 0/47 0/48 8/48 ^{§§(4+4)} [§]	0/48 0/47 0/48 4/48 (4+0)	0/48 0/47 0/48 14/48 (8+6)	0/48 0/47 1/48 6/48 (1+5)	≥0.02%: hyperplasia of transitional-cell epithelium in renal pelvis, ureters &	at 2%: hydronephrosis,	NCI 1977

Substance	Admini- stration route	Duration of treatment	Dose NTA % in DW or diet (mg/kg/d)	Urinary tract neoplasms ^a				Hyperplastic lesions	Nephrotoxicity	Reference
				Renal Tubular-cell neoplasms	Renal Transitional- cell epithelium neoplasms	Ureter Transitional-cell epithelium neoplasms	Bladder Transitional-cell epithelium neoplasms			
			2.0% (921 mg/kg/d)					urinary bladder, dysplasia of transitional-cell epithelium in ureter & urinary bladder at 2%: tubular cell hyperplasia in kidney		
Na ₃ NTA	Diet	18 mo + 6 mo recovery	0.0% 0.75% (355 mg/kg/d) 1.5% (724 mg/kg/d)	0/40 1/99 ^{'''} (1+0) @ 1/99 (1+0)	0/40 0/99 0/99	0/40 1/99 (1+0) 1/99 (1+0)	0/40 4/99 (0+4) 2/99 (0+2)	≥0.75%: hyperplasia of transitional-cell epithelium in renal pelvis, ureter & urinary bladder 1.5%: tubular cell hyperplasia in kidney	≥0.75%: higher incidences of chronic inflammation	NCI 1977
NTA	Diet	18 mo + 6 mo recovery	0% 0.75% (526 mg/kg/d) 1.5%	0/40 1/100 (1+0) 6/99 (5+1)	0/40 0/100 1/99 (0+1)	0/40 0/100 3/99 (3+0)	0/40 2/100 (0+2) 12/99 (0+12)	≥0.75%: hyperplasia of transitional-cell epithelium in ureter & urinary	≥0.75%: higher incidences of chronic inflammation	NCI 1977

Substance	Admini- stration route	Duration of treatment	Dose NTA % in DW or diet (mg/kg/d)	Urinary tract neoplasms ^a				Hyperplastic lesions	Nephrotoxicity	Reference
				Renal Tubular-cell neoplasms	Renal Transitional- cell epithelium neoplasms	Ureter Transitional-cell epithelium neoplasms	Bladder Transitional-cell epithelium neoplasms			
			(1053mg/kg/d)					bladder, tubular-cell hyperplasia in kidney		
Na ₃ NTA _z	Diet	28 wk	0% 0.3% 0.5% 1%	nd	nd	nd	-	0.3%: 13/21, 0.5%: 17/21, 1%: 19/21 of simple hyperplasia/uri- nary bladder	nd	Kitahori et al. 1985
Na ₃ NTA _z	Diet	28 wk	0% 1%	nd	nd	nd	-	1%:11/15: simple hyperplasias in urinary bladder	nd	Kitahori et al. 1988
Na ₃ NTA _z	Diet	28 wk	0% 1%	nd	nd	nd	-	1%: 7/19 simple hyperplasia of the urinary bladder	nd	Hiasa et al. 1985a
Na ₃ NTA _z	Diet	30 wk	0% 0.3% 1%	-	-	nd	nd	≥0.3%: dose- related increase of hyperplasia	at 3%: hydro- nephrosis & marked cystic	Hiasa et al. 1985b

Substance	Admini- stration route	Duration of treatment	Dose NTA % in DW or diet (mg/kg/d)	Urinary tract neoplasms ^a				Hyperplastic lesions	Nephrotoxicity	Reference
				Renal Tubular-cell neoplasms	Renal Transitional- cell epithelium neoplasms	Ureter Transitional-cell epithelium neoplasms	Bladder Transitional-cell epithelium neoplasms			
			3%					of renal tubules	dilatation	
Na ₃ NTA _z	Diet	32 wk	0.05% 1%	-	-	nd	nd	≥0.05%: dose- related hyperplasia of transitional-cell hyperplasia in renal pelvis at 1%: atypical cell foci of renal tubules	-	Hiasa et al. 1984
Na ₃ NTA _z	Diet	32 wk	2%	nd	nd	nd	-	No histological change in urinary bladder	nd	Fukushima et al. 1985

Summary of results from medium and long-term/cancer studies on mice

Table 4.1.2.8.2.B Urinary tract histomorphology

Substance	Adminis- tration route	Duration of treatment	Dose (% in DW or diet)	Urinary tract neoplasms ^a				Hyperplastic lesions	Nephrotoxicity	Reference
				Renal Tubular-cell neoplasms	Renal Transitional-cell epithelium neoplasms	Ureter: Transitional- cell epithelium neoplasms	Bladder Transitional-cell epithelium neoplasms			
Na ₃ NTA	Diet	18 mo + 3 mo recovery	0% 0.25% (169 mg/kg/d) 0.50% (338 mg/kg/d)	0/38 0/94 0/97	0/38 0/94 0/97	0/38 0/94 0/97	0/38 0/94 0/97	-	≥0.25%: hydronephrosis	NCI 1977
H ₃ NTA	Diet	18 mo + 3 mo recovery	0% 0.75% (752 mg/kg/d) 1.50% (1504 mg/kg/d)	0/40 5/88 (5+0) 27/94 (23+4)	0/40 0/88 1/94	0/40 0/88 0/94	0/40 0/88 0/94	≥0.75%: transitional-cell epithelium hyperplasia in the ureter and pelvis	≥0.75%: hydronephrosis at 1.5%: renal tubular degeneration & inflammation	NCI 1977
Na ₂ HNTA	DW	26 wk (5ml/d, 5 d/wk) + 10 wk recovery	0% 0.5% 0.5%	1/76 1/74 0/76	-	-	0/76** 0/74 0/76	-	-	Greenblatt & Lijinsky 1974
H ₃ NTA _Z	Diet	12 weeks	0% 1%	-	-	-	-	1%: renal hyperplasia in 1/10 mice	-	Matsuki et al. 1992

Explanations

a Total number of benign and malignant tumors, percentages were not reported since some of the tumor-bearing animals had multiple tumors in the urinary tract. Bilateral tumors at the same target site were counted as one tumor, but tumors at two sites in the same animals were counted separately (e.g. 1 tubular cell carcinoma and 1 transitional cell carcinoma in the renal pelvis of one animal were counted as two tumors).

@ (1+0) tumors in males and tumors in females

§ No. of animals affected/total no. of animals

DW: Drinking-water

§§ Kidney tumors not differentiated into localisations and/or types

nd: no data

§ In parentheses: no. of males + no. of females affected

-: no tumors observed

† In combination with 0.2% NaNO₂.

“’”: plus 1 hemangioma of the kidney

Combination with 0.1% NaNO₂.

“””: plus 1 haematoma of the kidney

** plus 1 (glom)angioma of the urinary bladder

∞ Data from groups with initiation treatment were not considered in the table.

Tumors in the urinary tract

In rats and mice, long-term treatment with NTA was associated with tumor development in the urinary tract. Oral 2-year studies in rats revealed tubular cell neoplasms and transitional cell neoplasms in the kidneys, and transitional cell neoplasms in the ureters and urinary bladder. In the mouse, the prominent types of tumors originated from the renal tubular cell (see Table 4.1.2.8.2.A+B). The minimum effective concentration that caused increased incidences of tumors in the urinary tract with origin from the tubular cell epithelium was 0.1% NTA within the drinking water (100 mg/kg/d) (Goyer et al., 1981). NTA-related increase in tumors with origin from the transitional cell epithelium started at concentrations of 7500 ppm NTA with diet that corresponded to 355 mg/kg bw/d $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ in rats (NCI, 1977). Comparing tumor response between species, the mouse was less sensitive than the rat where NTA-associated tumors occurred at lower dose, at more tumor sites and with a higher variability in tumor spectrum.

In rats, numerous adenomas and carcinomas of the transitional cell epithelium were observed in the renal pelvis (8%), the ureters (29%) and the urinary bladder (13%) of animals (males & females) that received diet containing NTA concentrations of 20000 ppm (921 mg/kg bw/d) for 24 months (NCI, 1977). Increased incidences in transitional cell epithelium tumors of the ureter (3%) and the urinary bladder (12%) were also seen after administration of diet concentrations of 15000 ppm NTA (1053 mg/kg bw/d) during 18 months followed by a 6-month recovery period (NCI, 1977). A single papilloma of the urinary bladder was seen at 2000 ppm (92 mg/kg bw/d). This tumor may be interpreted to be treatment-related, because of the absence of urinary tract tumors in the study controls, the knowledge of very low spontaneous incidences in rats and a high rate of urinary bladder tumors at the high dose. Preneoplastic lesions such as transitional cell hyperplasia and dysplasia of the renal pelvis, the ureter, and the urinary bladder occurred at all dose levels tested including the lowest tested of 200 ppm (9 mg/kg bw/d). No tumors at these locations have been observed in control animals of both studies.

Renal tubular cell neoplasms resulted from NTA administration with the drinking water at a concentration of 0.1 % (100 mg/kg/d) (Goyer et al., 1981) and within diet at a concentration of 20000 ppm (921 mg/kg/d) (NCI, 1977). Increased incidence or severity grades of tubular cell hyperplasias considered as preneoplastic lesions were reported for both studies. Tumor incidences of total numbers of tubular cell neoplasms were 15.8% (controls 2.7%) in the Goyer study and 16.6% (controls 0%) in the NCI study.

In mice, kidney tumors in male and female mice have been observed at NTA doses of 7500 and 15000 ppm (725 and 1500 mg/kg/d) after 18 months consumption with feed followed by 6 months of recovery (NCI, 1977). The kidney was the only tumor site, where numerous tubular cell neoplasms (6% and 29% at low and high dose, respectively) and a single tumor at the renal pelvis occurred. No tubular cell hyperplasia were seen in treated animals, and single transitional epithelium hyperplasias were seen in the renal pelvis and ureter of one low and one high dose mouse. Control animals did not develop tumors in the urinary tract.

The medium-term initiation-promotion studies of Kitahori and co-workers (1985, 1988) on the urinary bladder as well as the initiation-promotion studies on the kidney effects of Hiasa et al. (1984, 1985a, 1985b) on rats and mice (Matsuki et al., 1992) succeeded to demonstrate the acceleration of the tumor development initiated by nitrosamines. This does not necessarily indicate that NTA exclusively show tumor-promoting activities. Beyond the promotive action demonstrated, these studies were able to show that $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ and H_3NTA (without initiation) induced high numbers of hyperplasias in the urinary bladder and the kidneys after

relatively short time periods (28-30 weeks) related to the rat life time. As tubular hyperplasias were frequently observed in tumor bearing animals of conventional carcinogenicity studies, they are considered to represent interim stages in the development of renal tumors which originate from the tubular epithelial cells. The hyperplasias observed in the non-initiated NTA-treated groups of the initiation-promotion studies give support on the view of a genuine carcinogenic potential of NTA.

Tumors at other organs

A drinking water study with Na₂NTA on the development of lung tumors was negative after 26 weeks of treatment followed by a 10-week period of recovery (Greenblatt & Lijinsky, 1974). Here, treatment period might have been too short. A mouse cancer study with subcutaneous injection of NTA did not reveal higher rates of skin tumors (Van Duuren et al., 1974).

In one of the 18-months studies on NTA (NCI, 1977), there were higher tumor incidences in the livers, lungs and the adrenals of animals which were treated with NTA compared to those of the control groups. As the control animals presented a relatively high rate of spontaneous tumors at these organs, and equivalent findings were not reported by other studies, it was considered that their association to the treatment is inconclusive.

4.1.2.8.3 Considerations on the mode of action and conclusion

Carcinogenicity studies demonstrated that NTA is carcinogenic to rats and mice. The urinary tract was the target site of tumor development consisting of multiple types of primary tumors at several localisations. In rats, neoplasms of the kidneys originated from the tubular-cell epithelium and from the pelvic transitional epithelium. The transitional epithelium was also a target at the ureters and the urinary bladder (see Table 4.1.2.8.2.A). In mice, the target organ was the kidney where numerous tubular cell neoplasms and a single tumor with origin in the renal pelvis were found (NCI, 1977). The relative potency for carcinogenicity was higher in the rat than in the mouse. The lowest dose with treatment-related increased tumor rates in the urinary tract was 0.1% Na₃NTA (100 mg/kg bw/d) for the rat (Goyer et al., 1981) and 0.75% NTA (752 mg/kg bw/d) for the mice (NCI, 1977).

NTA tumorigenicity in the urinary system was not limited to a single renal cell type, e.g., those with primary excretion functions. Since tumors occurred at multiple sites along the excretion route (kidney, ureter, urinary bladder) and with origin from diverse target cells irrespective of their specific functions on excretion and reabsorption, this provides evidence that carcinogenic properties of NTA may be mediated by a direct cell contact to NTA-containing primary and secondary urine. The absence of NTA-associated lesions in the renal glomerula where ultrafiltration of plasma produced the primary urine can be interpreted that either NTA does not affect glomerular cells or that the form of NTA that is responsible for toxicity and carcinogenicity has not yet been produced. The primary site of the beginning of NTA lesions was the proximal tubule which is also the location where in healthy individuals organic ions were secreted.

Presumed mode of action

From the whole amount of data available, it was concluded that there is no plausible evidence for in vivo mutagenicity of NTA and its sodium salts.

Thus, it was postulated that other mechanisms than direct genotoxic action may be involved. It was hypothesized that the carcinogenic potential of NTA is related to sustained stimulation of cell proliferation following cytotoxic effects. To elucidate the likeliness of this assumption, the dose-relationship and time-relationship between tumors, preneoplastic changes and presumed initial lesions as the primary cause of regenerative cell proliferation should show corresponding features. The following paragraphs try to elucidate these issues.

Comparison of the dose-relationship of tumors and preneoplastic lesions

Multiple NTA-cancer studies in rats and mice showed higher numbers of hyperplastic lesions at several sites of the urinary system compared to their low numbers or absence in the controls (Tables 4.1.2.8.A and 4.1.2.8.B). Some of the rat cancer studies showed that the increase of urinary tract tumors was accompanied by hyperplasias at the target epithelium of tumor growth. Hyperplasias were found at the same doses like that at which tumors occurred (NCI, 1977; Goyer et al., 1981) or at lower doses without tumor response. The lowest effective dose inducing hyperplasias but no tumors was 0.02% Na₃NTA in diet (≈ 9 mg/kg/d, 24 months, NCI, 1977) (Cave: one single tumor in urinary bladder at this dose). This provides some evidence that the hyperplasias of the tubular-cell epithelium (kidney) and of the transitional epithelium (renal pelvis, ureter, urinary bladder) were the evolutionary preneoplastic lesions.

However, not all rat studies were able to demonstrate this association. For example the 18-months treatment with Na₃NTA.H₂O of rats (NCI, 1977) yielded tumors (neoplasm of renal tubular cell epithelium and ureter) at both doses tested, but the corresponding hyperplasias were only seen at the high dose. This might be interpreted as a possible effect of recovery, since the animals lived until the scheduled end of a 3-month recovery time after treatment. In mice, hyperplasias occurred in transitional cell epithelium of ureter without any progression to tumors at these sites (see also Table 4.1.2.8.3.B). In contrast, hyperplasias as potentially preneoplastic lesion were not seen for the tubular cell neoplasms in mice (NCI, 1977).

Temporal association of presumed preneoplasia with the tumor development:

A short treatment period of 9 days at 1876 mg/kg bw/d Na₃NTA or 3 weeks at 1500 mg/kg bw/d H₃NTA was sufficient to induce basophilic/clear cell renal tubular hyperplasia in rats (Merski, 1982; BASF, 1997b). Hyperplasia of the pelvic transitional epithelium has been observed as early as 13 days after treatment begin (Merski, 1982). In a 4-week study (BASF, 1998), 20000 ppm Na₃NTA.H₂O (926 mg/kg bw/d) was efficient to cause hyperplasia of proximal tubules and renal pelvic urothel in all rats of this dose group.

A number of medium-term studies in which NTA-only treated rat groups served as controls for initiation-promotion tests showed that oral administration for 28 to 30 weeks resulted in higher numbers of hyperplasias of the renal tubule and in the urinary bladder (Kitahori et al., 1985, 1988; Hiasa et al., 1984, 1985b).

The latency period of tumor occurrence was not documented in the NCI cancer studies (1977). The survival rates were comparable between the treated and control animals except

the high dose rats (20000 ppm Na₃NTA.H₂O) from the 24-month study. Using the survival curves as an indirect indicator on tumor-associated mortality, the high survival rates at the end of the studies gave hint for the assumption that the urinary tract tumors represented late life tumors.

These data support the assumption that hyperplasias were the corresponding preceding lesions in the tumor development. However, there are some data gaps and inconsistencies as outlined in the section "Strength and consistency".

Role of cytotoxicity as initial lesion in tumor development:

Ideally, if cytotoxicity is the critical precursor lesion in neoplastic growth it is expected to occur at lower doses and to begin after shorter latency period than preneoplasias or tumors do. Increased rates of cell proliferation may result by chance in disregulated cell growth and replication and thereby enhance a multistep progression to tumor.

NTA-related tumors already occurred at a dose of 100 mg/kg/d (704 d, see Goyer et al., 1981). This dose was in the range of the lowest dose of 97 mg/kg/d that was shown to be cytotoxic (LOAEL 97 mg/kg/d, 6 mo, Nixon et al., 1972). That means carcinogenic potential of NTA was not restricted to extremely high concentrations far above toxic doses. In favour of the postulated mode, this low tumor effective dose might be explained as an effect of treatment duration.

Cytotoxic events in the target cells were degeneration, vacuolisation of epithelia and/or hydronephrosis observed in multiple animal studies. Vacuolisation of the proximal tubular epithelium was an early degenerative lesion occurring already after nine days of treatment (Merski, 1982). It was accompanied by increased incidences of reactive interstitial inflammatory in the renal cortex at 4 weeks of treatment (BASF, 1998). Cytotoxicity occurred at the same dose levels as hyperplasias (Merski, 1982; BASF, 1998) or at lower doses (BASF, 1997b). Beside the vacuolation of tubular cells, basophilic appearance of tubules has been observed in both studies. This lesion is considered to give evidence of increased regenerative activity and may represent a precursor lesion of basophilic tubular hyperplasia.

Hydronephrosis is a synonym for the enlargement of renal pelvis, and is considered as a result from preceding cytotoxic effects in the papillary area and/or pelvic epithelia. It has been observed in short term toxicity studies (BASF, 1998) as well as in some long term/cancer studies. It was described in the 24-months study on rats (NCI, 1977) at the high dose at which renal pelvic tumors were seen. In contrast to this, the occurrence of one tumor of the renal pelvis at the top dose of the 18-months NTA study on rats (NCI, 1977) was not corroborated by hydronephrosis. This observation may be related to the 6 months of recovery, but other studies demonstrated the nonreversibility of hydronephrosis (Myers et al., 1982). In mice, (pelvic) hydronephrosis was observed at doses ($\geq 0.75\%$ H₃NTA in diet; ≈ 752 mg/kg/d) that induced renal tubular tumors (NCI, 1977); only at the high dose (1.5% H₃NTA) one tumor of the pelvic region was found.

Pelvic dilatation was reported to parallel with the severity of hyperplastic lesions in this area and with epithelial loss or necrosis of the renal papilla (BASF, 1998). This corresponded to the observation of Merski (1982) who found from day 13 onwards necrosis, hemorrhages and hyperplasia of the pelvic transitional cell epithelium. Synonymously, Alden et al. (1981) interpreted the epithelial hyperplasia as a sequelae to the epithelial erosion and ulceration of the pelvic transitional epithelium. The degenerative lesions in the pelvic urothelium were

accompanied by hemorrhagic, inflammatory and fibrotic lesions in the epithelium and the subepithelial medullary area (Alden et al., 1981; BASF, 1998; Myers et al., 1982).

Strength and consistency

Tables 4.1.2.8.3 A and B summarise the presumed sequential steps of NTA-related effects separately for each tumor site. Consistence between target sites of cytotoxicity, preneoplastic lesions and tumor growth was observed for most localisations in the rat (Table 4.1.2.8.3.A). The existence of cytotoxicity at most tumor sites (except urinary bladder) provides evidence of being the tumor-associated precursor lesion. Time-response relationships as demonstrated for a number of rat studies support this assumption. The data support the hypothesis that cytotoxicity induced regenerative hyperplasia that preceded the tumor growth. Therefore, it seems to be plausible that one initial step in the tumor development was linked to the cytotoxic effect of NTA.

In general, mouse data (Table 4.1.2.8.3.B) were in line with the observations from rat. Due to limited data base for mice, a coincidence of cytotoxic and carcinogenic effects was only given for the renal tubular cell epithelium. Hyperplastic changes have not been observed in the renal cortex, the most relevant tumor site in mice, but were seen at other target sites (renal pelvis and ureter). The presence of hyperplasia in the ureter gave some concern that carcinogenic potential of NTA may not be restricted to the kidney for the mouse if the animals would have been treated for more than 18 months.

In addition, there are some inconsistencies and data gaps.

Data from subacute toxicity studies in rats revealed that vacuolar degeneration was associated to NTA treatment (Alden et al., 1981; BASF, 1998), but not strongly linked to hyperplasia of tubules. Beside vacuolar degenerated areas and vacuolar hyperplasias of the renal tubules, there were (basophilic) hyperplastic lesions without any vacuolisation (BASF, 1998). Merski (1982) reported that the majority of nodular hyperplasia was associated with vacuolar degeneration of tubular cells, whereas less than 10% were of basophilic appearance. It remains unclear whether the cytotoxicity was a necessary condition for the development of all types of hyperplasias. Alternatively, hyperplasias might also develop as an early prestage of a tumor independently from cytotoxicity.

It may be speculated for cancer bioassays where no or only few indications of cytotoxicity were found that cytotoxicity might have been a transient effect, that was not longer evident at the end of long-term studies. In contrast to multiple studies that described cytotoxicity on different segments of the nephron as a treatment-related effect, this may be the case in the study of Goyer et al. (1981) which did not report any indication on cytotoxicity at the tumor-inducing dose after 704 days of treatment.

In another example, hydronephrosis at the high dose (921 mg/kg/d) was the only toxic effect seen after 24 months in the NCI study (NCI, 1977), whereas preneoplasia such as hyperplasia and dysplasia of the transitional cell epithelium were seen at 9 mg/kg/d and above unaccompanied by any cell damage. In this case, no dose-response could be estimated since an underlying toxic effect was absent.

For the renal cortex compartment, short term studies clearly demonstrated the evidence of cytotoxicity as an early lesion associated with hyperplastic response. But, no associated cytotoxic precursor lesion was identified for the urinary bladder in the rat and the mouse. This

may be due to data lack on these specific tissue sites, since tissue samples of the urinary bladder were not routinely examined by histomicroscopy.

Also, data on NTA effects on the ureter were sparse. Erosion of the urothelium was reported in a rat 4 week study of Kanerva et al. (1984), macroscopic thickening may be indicative for a hyperplastic response. No data were available on the existence of degenerative and/or inflammatory lesions or tumors in the ureter for the mouse.

Table 4.1.2.8.3.A: Target sites and effects in the rat urinary system after repeated oral administration of NTA

Target site	Renal tubule	Renal pelvis	Ureter	Urinary bladder
Target cells	Tubular-cell epithelium	Transitional-cell epithelium		
Precursor lesion	Cytotoxicity: Vacuolation, Degeneration of proximal tubules 1,2,4,6,7,8,9	Cytotoxicity: Necrosis, Erosion, Ulceration, Hemorrhage, ^{2,3,4, 6} Hydronephrosis ^{3,4,5,6,7,10,16}	Cytotoxicity: Dilation ^{3,5} Erosion ⁵	<i>No data/not reported</i>
Preneoplasia	Hyperplasia NOS* ^{10,12, 16} , Vacuolated cell hyperplasia ^{1,2,3,4,6} , Basophilic cell hyperplasia ^{1,2, 6} , Clear cell hyperplasia ¹	Hyperplasia ^{2,3,4,6,10,12,17}	Hyperplasia ^{5,10,11,12}	Hyperplasia ^{10,11,12,13,14,15}
Atypia/Dysplasia	Atypical cell foci ¹⁷	Dysplasia ⁶	Dysplasia ¹⁰	Dysplasia ¹⁰
Neoplasia	Tumors ^{10,11,12,18} Tubular-cell adenomas Tubular-cell adenocarcinomas	Tumors ^{10,11} Transitional-cell papillomas Transitional-cell carcinomas	Tumors ^{10,11,12} Transitional-cell papillomas Transitional-cell carcinomas	Tumors ^{10,11,12} Transitional-cell papillomas Transitional-cell carcinomas Squamous cell carcinomas

Data sources: ¹ BASF, 1997b, ² Merski, 1982 ³ BASF, 1998 ⁴ Anderson & Kanerva, 1979, Alden et al., 1981 ⁵ Kanerva et al., 1984, ⁶ Myers et al., 1982, ⁷ Nixon, 1971, ⁸ Nixon et al., 1972, ⁹ Mahaffey & Goyer, 1972, ¹⁰ NCI, 1977 (24 mo), ¹¹ NCI, 1977 (18 mo, NTA), ¹² NCI, 1977 (18 mo, Na₃.NTA), ¹³ Kitahori et al., 1988, ¹⁴ Kitahori et al., 1985, ¹⁵ Hiasa et al., 1985a, ¹⁶ Hiasa et al., 1985b, ¹⁷ Hiasa et al., 1984, ¹⁸ Goyer et al., 1981

* NOS not otherwise specified

Table 4.1.2.8.3.B: Target sites and effects in the mouse urinary system after repeated oral administration of NTA

Target site	Renal tubule	Renal pelvis	Ureter	Urinary bladder
Target cell	Tubular-cell epithelium	Transitional-cell epithelium		
Precursor lesion	Cytotoxicity: Tubular degeneration ¹	Cytotoxicity: Hydronephrosis ¹	<i>No data/not reported</i>	<i>No data/not reported</i>
Preneoplasia	<i>Hyperplasia not reported</i>	Hyperplasia ¹	Hyperplasia ¹	<i>No data/not reported</i>
Neoplasia	Tumors ¹ Tubular-cell adenomas Tubular-cell adenocarcinomas	Tumor? ¹ Transitional-cell papilloma [#]	<i>No data/not reported</i>	<i>No data/not reported</i>

Data source: ¹ NCI, 1977, [#] only 1/94 mice

In conclusion, there is sufficient evidence that NTA (cyto-)toxicity on the urinary tract is followed by regenerative cell proliferation (hyperplasia) and tumor development. The evidence is supported by:

- Comprehensive consistence of target sites in the rat urinary system where NTA treatment induced cytotoxicity, hyperplasia/dysplasia or neoplasia.
- There are some indications on consistency of target sites in the mouse urinary system.
- There is sufficient evidence that cytotoxic effects of NTA were followed by regenerative hyperplasias in a dose- and time-related manner.
- There is sufficient evidence for time-response relationship of hyperplasias and tumors.
- There is some evidence for dose-response relationship of hyperplastic lesions and tumor developed within the studies available.

However, some points limit the strength of evidence:

- There are some indications that hyperplasias/dysplasias can also develop without preceding cytotoxic events.
- The strength of evidence was limited by the fact that the overall LOAEL for toxic effects (97 mg/kg/d, Table 4.1.2.6.2) based on all studies considered relevant was identical to the lowest tumor dose (100 mg/kg/d., Table 4.1.2.8.2.A).
- There are data gaps for some target sites (urinary bladder, ureter) and especially for data from the mouse.

- It remains to be clarified whether cytotoxicity was a sufficient condition for tumorigenesis. Comparing to other xenobiotic substances inducing similar cytotoxicity without having a carcinogenic potential, additional factors must contribute to the NTA carcinogenesis.

Remaining uncertainties

Even if it seems likely that cytotoxicity is involved in the tumorigenesis of NTA, uncertainty remains whether it represents the crucial causal factor in tumor initiation or whether there are other causal factors. Due to the ability of NTA to form complexes with several metal cations that are ubiquitous in cells of the mammary organism it might be that NTA exert its carcinogenic action through the endogenous formation of metal complexes. This formation of water-soluble chelate complexes can occur at all sites during the absorption, distribution and excretion of NTA. Due to their high complex binding constants at neutral or weak acid pH values iron (as Fe^{3+}), copper and zinc may preferably bind to NTA (Becke-Göhring & Fluck, 1961). The amount and proportion of individual metal ion NTA complexes (e.g. ZnNTA, FeNTA) is thought to be dependent on the concentration of metal ions at different tissue sites (serum, cytosol, urine) and the strength of the binding in metal protein complexes in situ.

Contribution of zinc

Repeatedly, NTA toxicity was discussed to be mediated by zinc ions bound to NTA resulting in elevated zinc excretion with the urine. The following data on altered zinc homeostasis were found in the studies available.

A four week oral administration of high NTA doses (≥ 926 mg/kg/d) revealed an increased excretion of urinary zinc in rats whereas normal concentrations were found at low concentration of appr. 9 mg/kg/d (BASF, 1998, Bahnemann et al., 1998, Michael & Wakim, 1973). A lower dose of 239 mg/kg/d of Na_3NTA given with the drinking water for 35 days had no effect on kidney or plasma concentrations of zinc (Krari & Allain, 1991). Increased zinc concentrations in serum were seen in some rat groups at 0.15% Na_3NTA or above (97 mg/kg/d, 6, 12, 18, 24 months), but increase was not persistently found at all time intervals examined (Nixon et al., 1972). In mice, oral administration of 100 mg/kg NTA (1/4 LD₅₀) had no effect on urinary elimination of zinc or other metals (Fe, Cu, Mn) (Cantilena and Klassen, 1982). Urinary zinc values were raised in dogs fed on 90 days a NTA containing diet (up to 130 mg/kg/d), but were not associated to histomorphologic findings in the kidneys. They showed high individual variability and a lack of dose-relationship (Budney et al., 1973).

At present, the evidence on dose-related increase in urinary zinc excretion by NTA exposure is insufficient. Data supporting dose-related effects of NTA at doses below 926 mg/kg/d on urinary zinc excretion are missing. The increased zinc concentration in urine at 926 mg/kg/d should result in an imbalance of zinc homeostasis of which the toxicological significance for carcinogenicity is unknown.

It might be that ZnNTA complexes are formed at NTA doses below those resulting in urinary zinc excretion. Thus ZnNTA complexes may exert their toxicity at target sites of tumor production and thereby contribute to NTA carcinogenicity. This must not necessarily be associated with tissue zinc accumulation, increased zinc levels in serum, tissues and urine. A contribution of zinc in the process of NTA tumor development can not be excluded, however, based on the present knowledge, a firm conclusion cannot be drawn.

Contribution of iron

Depending on the pH conditions in body fluids or tissue cells, the relationship of formed metal complexes may be different. Because iron is the most abundant transition metal in the human body and the high complex binding constant with NTA the formation of FeNTA complexes is expected.

In theory, FeNTA may be generated before or after the glomerular filtration process. Assuming that NTA may exert its toxicity/carcinogenicity from the luminal side of the nephron within the weakly acidic urine, it is expected that the generation of FeNTA complexes is significantly higher than that of ZnNTA complexes (Leibold et al., 2002).

At present, the knowledge is insufficient for evidence that NTA metal complexes are formed in situ. It needs further investigations on the site of their formation, their toxic effects and its contribution to the initial tumor development or their interference with the cytotoxicity by NTA.

Conclusions

- NTA is a transspecies carcinogen. It is carcinogenic in both sexes of two species, the rat and the mouse.
- Carcinogenicity was demonstrated for the oral route. No or sparse data were evident for the other inhalative or dermal routes.
- NTA is not metabolised and exerts its carcinogenic activities via the urinary excretion route.
- At several localisations in the urinary tract NTA induced primary tumors. Multiple tumor types were observed. Tumors in the rat kidney originated from the tubular-cell epithelium and from the pelvic transitional cell epithelium. Tumors from the transitional cell type were also found in the ureter and the urinary bladder. For the mice, tumors originated from the renal tubular epithelium and occasionally from the renal pelvis.
- Based on the actual knowledge a direct genotoxic mechanism of NTA carcinogenesis could not be demonstrated. At present, it is thought that NTA might be operative at target sites by other threshold-based actions.
- It seems to be reasonable that NTA related cytotoxicity plays a crucial role in the development of tumors. The facts that the cytotoxicity and tumors were seen in identical target regions of the kidneys, that cytotoxicity is an early lesion that leads to regenerative hyperplasia and that hyperplasia was often associated to tumor growth provide evidence for this mode of action.
- Beside the sequential cascade of morphological events – cytotoxicity, hyperplasia/dysplasia, neoplasia – other factors might contribute to disregulated cell growth and to the manifestation of neoplastic cell growth either as initial events before obvious cytotoxicity or in parallel to the cascade from cytotoxicity to tumor. Interference with metal cations and forming of metal complexes might be suspected. However, the data presently available were insufficient to give sufficient evidence for their contribution in the NTA carcinogenicity.

- Finally, the exact mechanisms of carcinogenic effect remain unclear.
- The effect must be assumed to be relevant for humans.
- The substance needs to be classified as a category 3 carcinogen, and the labelling with R40 (Limited evidence of a carcinogenic effect) is required.

4.1.2.8.4 Estimation of a starting point for quantitative risk characterisation on nongenotoxic carcinogenesis of NTA

Before selecting an appropriate starting point for quantitative risk characterisation, the carcinogenicity study with the most sensitive critical effect, that means the treatment-related tumor response at the lowest dose, was identified. The 24 months-study in rats with diet application of Na₃NTA (NCI, 1977) was chosen due to its treatment duration, lowest effective tumor dose (92 mg/kg) with a significant increase in tumors (see Table 4.1.2.8.2A). In this study, increases in tumor incidences were seen at multiple localisations (renal tubules, renal pelvis, ureter, urinary bladder). Since transitional cell hyperplasias, dysplasias and tumors were considered to represent a sequence of events in a multi-stage tumor growth, their incidences were summarised to determine the localisation with the highest incidences of pre-/neoplastic lesions. According to Table 4.1.2.8.1 the most sensitive localisation was the urinary bladder; the most sensitive sex was the female rat. The total numbers of preneoplastic and neoplastic events at this site are repeated in Table 4.1.2.8.4.

Table 4.1.2.8.4 Summary on incidences of hyperplasias, dysplasias and tumors of the transitional cell epithelium in the urinary bladder of rats (extract from Table 4.1.2.8.1)

Dosis mg/kg (% test substance in diet)	Control group		9 mg/kg/d (0.02%)		92 mg/kg/d (0.2%)		921 mg/kg/d (2%)	
	24 m	24 f	23 m	24 f	24 m	24 f	24 m	24 f
No. of animals/ sex examined:								
Urinary bladder								
TC hyperplasia	0	0	3	1	3	13	8	14
TC dysplasia	0	0	1	1	4	3	0	8
TC tumor	0	0	0	0	0	1	1	5
Σ total no. of preneoplasias & neoplasias	0	0	4	2	7	17	9	27* (24)

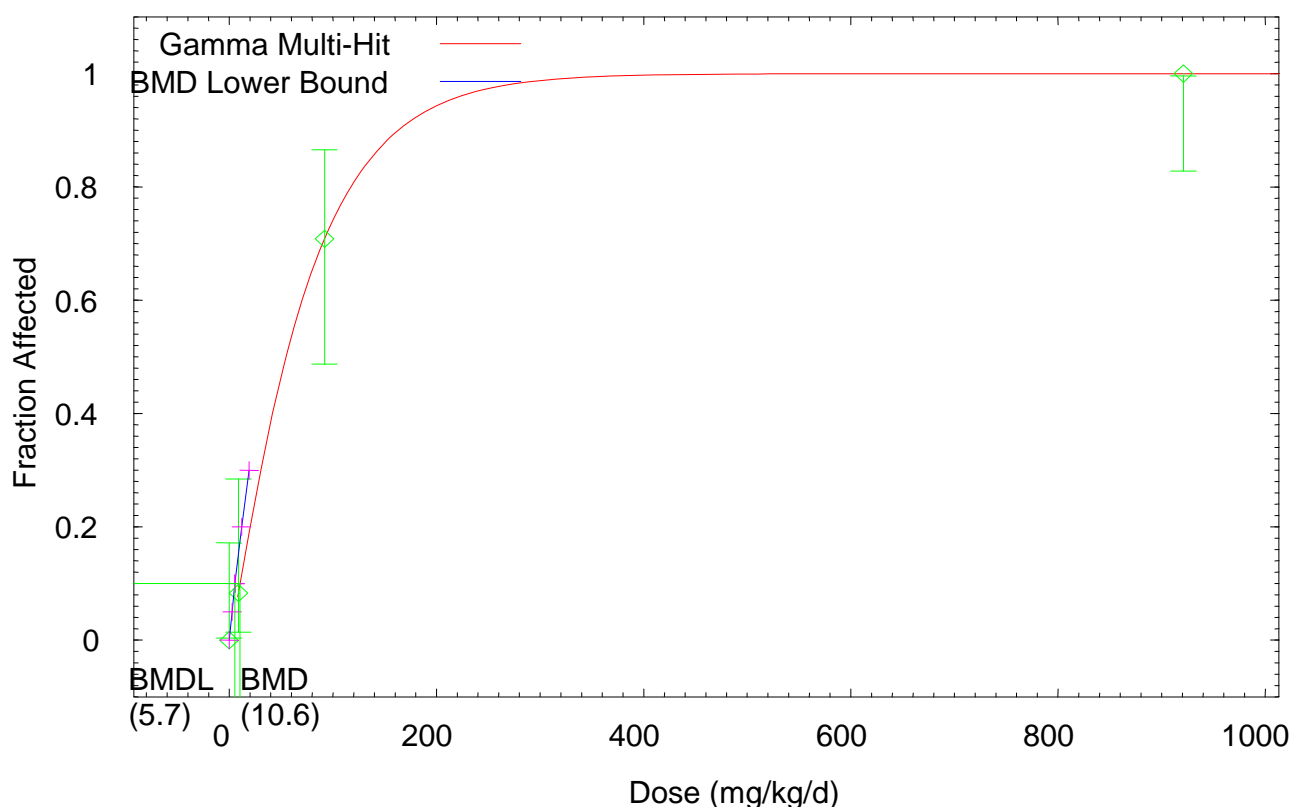
In the dose-response assessment of NTA, a starting point for quantitative risk characterisation can be identified by two options: the benchmark dose (BMD) approach and the NOAEL/LOAEL approach.

The numbers of preneoplastic (TCC hyperplasia and dysplasia) and of neoplastic (TCC tumor) were selected as the benchmark response. Since no individual animal data are available in the NCI report it is generally assumed that each animal had only one lesion per urinary bladder. In the high dose group of females at least one animal carries multiple lesions

as the number of lesions (27*) exceeded the number of females treated. For the modelling it is assumed that 100% of high dose females were affected by at least one lesion, i.e. the corrected value of 24 were used for BMD calculations. This might overestimate the dose effect at the high dose level because it does not reflect when multiple animals had multiple lesions. However, this is thought to be of minor relevance for the calculation at the lower end of dose response range.

A benchmark dose was calculated using the U.S.EPA BMD software (Version 1.3.2). Data fitted best when the Gamma Multi-Hit model was applied (AIC criterium 46.7). By this model the benchmark dose lower bound (BMDL) where a treatment-related increase in response of 10% could not be expected within the 95% confidence interval on the BMD was 5.7 mg/kg bw/day. It is proposed to use this value as starting point for quantitative risk characterisation.

Benchmark Dose Calculation (Gamma Multi-Hit Model, 0.95 Confidence Level) on pre-neoplasias and tumors of the urinary bladder of NTA treated female rats



4.1.2.9 Toxicity for reproduction

Animal data:

Fertility impairment

Guideline-according generation studies for Na₃NTA are presently not available.

Limited data relating to reproductive performance were reported from a combined reproduction and teratology study (Nolen et al., 1971), where rats (Charles River CD) had been exposed via diet for two successive generations. Na₃NTA (chemical identity and purity not further specified) had been mixed to ground Purina Chow (contents and stability not further specified) to yield levels of 0.1 and 0.5% in the diet, equivalent to a daily intake derived for adult female rats of approximately 90 and 450 mg/kg bw/day. Dose levels had been chosen from previous subacute and long-term studies, from which the lower level had been reported to be without any effect, whereas the 0.5% level had been reported to produce some mild toxicity (not further specified). Groups of 20 male and female rats each were fed these two dose levels continuously throughout two generations. The animals were randomly caged individually in a carefully controlled environment and fed ad libitum. The original F0 generation parental rats were bred three times: litters from the first breeding trial were followed up until weaning when they were discarded, descendants from the second breeding provided the F1 generation parental animals, litters from the third breeding were used for teratology studies. The second-generation (F1) animals were bred twice with the first litters followed up until weaning and the litters of the second breeding used for teratology studies. For each generation records of individual weekly feed consumption and body weight gain were kept during the first 8 weeks from weaning. All live-born litters were counted at birth and again 4 days later, when the pups were weighed and their sex determined. After this, offspring were culled to eight pups per litter. The pups were weaned and again weighed at 21 days after birth.

Mean total food consumption (determined by week 8) at the high dietary level (0.5% Na₃NTA) was somewhat less than in the control and in the low dietary level (0.1% Na₃NTA) group in both sexes in the F0 generation and in the F1 males, statistically significantly different, however only for the F0 males. Also, mean body weight gain in male and female rats of both generations was slightly reduced in comparison to either controls or to the 0.1% group, statistically significantly different, however only for F0 females and for F1 males. There were no significant differences in food efficiency (body weight gain per food intake).

With respect to reproductive performance there were no significant differences in the conception rate during the specific phases of the study and no significant differences in the measures of fertility and lactation in terms of average numbers of live-borns per litter, live pups on p.n. day 4, average number of weaned pups per culled litter and in the lactation index. Body weights of the new-borns were not reported. Offspring weaning weights were reduced at the 0.5% level in both sexes, an effect observed in litters of the first breeding trials and statistically significant for the F1 generation only, but not consistent across successive breedings and/or across generations.

Developmental toxicity:

Guideline-according developmental toxicity studies for Na₃NTA are presently not available.

During the course of the above mentioned two-generation study in Charles River CD rats (Nolen et al., 1971) separate groups of animals derived from the third breeding trial of the F0 generation and from the second breeding trial of the F1 generation were used for additional teratological studies. Groups of 20 pregnant rats were exposed to diets containing 0.1 or 0.5% Na₃NTA during day 6 - 15 of gestation. Of each group ten dams were sacrificed on day 13 of gestation, respectively on day 21 of gestation. In dams sacrificed on day 13 of gestation the numbers of corpora lutea, implantations and resorptions were recorded. Fetuses removed from

dams sacrificed on gestation day 21 were inspected for gross abnormalities, weighed and their sex determined and further investigated for skeletal and visceral defects.

No differences were observed in the dams of either of the two treatment groups in comparison to controls with respect to food consumption and body weight gain. Dams sacrificed on day 13 of gestation did not differ significantly from those sacrificed at day 21 of gestation in the number of corpora lutea, implantations or resorptions. Overall, the treatment groups did not differ significantly from control groups with respect to average number of corpora lutea, resorptions, live or dead fetuses or in the average weight of male and female fetuses.

Fetal evaluation for abnormalities did not reveal any skeletal defects. Soft tissue defects predominantly in the urinary system (hydroureter and /or hydronephrosis) were detected, however, the control groups of both generations were affected as much as any of the experimental groups. The authors therefore suggested that these effects were attributable to some unknown agent, possibly a virus.

During the course of the study of Nolen et al. (1971), teratological studies were also performed on rabbits. Groups of 20 female New Zealand does were treated by gavage after artificial insemination with daily doses of 2.5, 25, 100, or 250 mg Na₃NTA in distilled water in a volume of 2 ml/kg bw during days 7 - 16 of gestation. One control group received water alone and another received no treatment at all. Except the untreated controls, all doses were weighed every three days during pregnancy so that dosages could be adjusted. Animals were sacrificed on gestation day 28 and the offspring were treated in a way similar to that described for the rat study.

Data on the investigation of maternal parameters (food consumption, weight gain) were not given. As a result there were no significant differences in the average numbers of corpora lutea, resorptions, live and dead fetuses or in the average fetal body weights between treatment groups and controls. Also, no significant differences were seen in the number with gross abnormalities, skeletal or soft-tissue defects.

Na₃NTA was further investigated by several groups for the interference with heavy metal toxicity and teratogenicity in rats (Cd, methyl-Hg).

Gavage studies with the coadministration of Na₃NTA at dose levels of 9.4 - 50.3 mg/kg bw/day to CH₃HgOH or at dose levels of 62.5 - 250 mg/kg bw/day to CdCl₂ in pregnant Charles River COBS rats during g.d. 6 - 19 did not reveal any effect, respectively enhancement of the extent or type of the teratogenicity of both of these metals (Scharpf et al., 1972, 1973).

Na₃NTA at dose levels of 0.1 or 20 mg/kg bw/day coadministered to CdCl₂ or CH₃HgOH via drinking water to pregnant Charles River CD rats during g.d. 6 -15 did not produce any change or increase in the incidence of Cd or Hg induced malformations. However, also in the groups given only Na₃NTA, there was an apparent increase in hydronephrosis and bladder defects. But, since these conditions had been observed by the authors also in other controls at significant and varying incidences (10 - 40%) this effect was discussed not to be attributed to Na₃NTA treatment (Nolen et al., 1972 a,b).

In a further study with mice the distribution and teratogenicity of nitrilotriacetic acid (NTA) was investigated (Tjälve, 1972). Two groups of pregnant and nonpregnant NMRI or C57 Black mice were given ¹⁴C-labelled NTA intravenously, respectively per os, and the distribution of radioactivity at different time-intervals was studied with whole-body autoradiography. Two additional groups of ten NMRI mice each were either given 0.2% NTA via drinking water during g.d. 6 to 18 or taken as controls. Dams were weighed daily and at

sacrifice on g.d. 18 evaluated for the numbers of resorptions and live fetuses. Fetuses were weighed and examined for external, skeletal and visceral defects.

The results from the autoradiographic study revealed a strong accumulation of radioactivity in the skeleton present up to 48 h, which was the longest time-interval studied. There was also a strong accumulation in the fetal skeleton thus indicating transplacental transfer.

During the course of application via drinking water, there were no significant differences in comparison to controls with respect to maternal weight gain during treatment, the numbers of resorptions, mean live fetuses (88 from controls/92 from treated) per litter or mean fetal weight. External, skeletal or visceral examination of the fetuses did not reveal any teratogenic effects.

Other information:

NTA has been further studied - mostly as a presumptive non-teratogenic reference compound - in a couple of *in vitro* systems proposed for screening for teratogenicity using embryonic tissues or embryos from mammalian and nonmammalian species.

In a validation study for the rat postimplantation whole embryo culture system (Cicurel and Schmid, 1988) NTA turned out to be teratogenic (specific malformations observed in $\geq 30\%$ of the embryos) at minimum concentrations of 300 μg NTA/ml culture medium and to impair differentiation (somite number) and growth (crown-rump length).

In studies with a preimplantation embryo culture system using murine 2-cell embryos developing until blastulation (Merlini et al., 1985) NTA was reported to reduce survival and to impair blastulation at medium concentrations of ≥ 0.01 mM.

In an *ex vivo-in vitro* test system developed by Flint and co-workers (Flint 1986; Flint and Orton 1984; Flint et al., 1984) pregnant Alderly Park (Alpk) Strain I rats were treated with a single dose of 250 mg NTA/kg bw on g.d. 12 by intra-peritoneal injection from which embryos of selected somite stages were harvested 16 h later and prepared for differentiation cultures of mid brain and limb bud cells. Growth (total protein) and progress of differentiation (incorporation of $^3\text{H-GABA}$ or $^{35}\text{SO}_4$) were evaluated in the respective micromass cultures 5 days later. Inconsistent test results were reported from these investigations. While in one report NTA was identified as a teratogen (Flint et al., 1984), the other reported NTA not to inhibit differentiation at any of the concentrations used (Flint and Orton, 1984).

NTA was also investigated *in vitro* within a validation exercise of a more improved micromass culture system using limb bud cells from Sprague-Dawley derived rat embryos (Renault et al., 1989). In this investigation NTA was identified as a borderline inhibitor for proliferation.

Within the course of a NTP study on the evaluation of two *in vitro* teratology cell culture test systems (NTP-PB87-146841 1986) NTA was investigated amongst 44 chemicals for growth inhibition in a human embryonic palatal mesenchyme (HEPM) cell line and for inhibition of cell attachment in primary mouse ovarian tumor (MOT) cells. NTA was positive in the two test systems with average IC_{50} values of 999 $\mu\text{g}/\text{ml}$ (3.53 mM), respectively 332 $\mu\text{g}/\text{ml}$ (1.07 mM). By means of certain cut-off values for the evaluation of the performance of the different chemicals tested, the findings for NTA in these two *in vitro* systems were considered to be in concordance with the *in vivo* data.

Furthermore, NTA was evaluated in a short term test proposed as a screening assay for chemical teratogens using mid-to-late blastula stage embryos from frogs (*Xenopus laevis*)

cultivated *in vitro* for 96 hours (Dawson et al., 1989; Sabourin and Faulk, 1987). Within these studies NTA was classified to have little or no teratogenic potential.

Within the evaluation of a further bioassay using fruit fly larvae (*Drosophila melanogaster*) NTA was evaluated negative for the induction of external structural malformations in this species (Lynch et al., 1991).

Human data:

No human data available.

Conclusion:

The available data from one older *in vivo* study in laboratory animals are not sufficiently detailed reported for a sound evaluation of the respective study. However, from available information of this study it appears that NTA at dietary levels of approximately 450 mg/kg bw/day did not adversely affect reproductive performance and capability through two successive generations in rats. Higher dose levels up to and including clearly parentally toxic doses have not been investigated so far. In addition, any specific teratogenic potential and/or impairment of embryo/fetal development was not indicated from the data of that study for rats at dietary levels of approximately 450 mg/kg bw/day and for rabbits at (gavage) doses of 250 mg/kg bw/day. Higher dose levels of NTA up to and including maternally toxic doses have not been investigated so far, however, it was demonstrated for mice that NTA probably passes the placenta and accumulates in the fetal skeleton. With respect to developmental toxicity, the negative findings from the study with rats and rabbits are further supported from the results of a drinking water study in pregnant mice and also from studies with heavy metals, where NTA was demonstrated not to interfere with heavy metal induced teratogenic effects in rats.

4.1.3 Risk characterisation

4.1.3.1 General aspects

In general, for the evaluation of systemic effects of trisodium nitrilotriacetate (Na_3NTA , abbr. NTA) studies with administration of H_3NTA or of its salts such as Na_2HNTA , NaH_2NTA and Na_3NTA were considered as relevant information because these compounds are dissociated under physiological conditions (pH 7-9) into the sodium cations and the respective anionic species of nitrilotriacetic acid depending on the pH-dependent dissociation equilibrium of H_3NTA . Taken together, any conclusions on Na_3NTA will be derived from consideration of the overall available data base.

NTA is well absorbed in dogs, rats and mice, but is poorly absorbed by humans after oral administration. The biological half-life for the elimination of NTA from the soft tissues of rats was approximately three hours. Studies in dogs, rats and mice indicated that NTA did not enter the enterohepatic circulation. The absorbed NTA was rapidly excreted via the urine except for a small fraction remaining in the bone. The urinary clearance is accomplished by passive glomerular filtration in rats. Any biotransformation of NTA was not observed in the studies for men, dog, rats and mice. Less than one percent of the administered dose was

excreted in the expired CO₂. Urinary calcium and zinc concentrations were increased in urine of rats and mice with high urinary NTA levels. The disposition of copper, iron and manganese was not appreciably influenced by oral administration up to 2% Na₂NTA or 1.5% NTA in diet to rats.

Based on the animal studies it may be appropriate to choose 50 % as a default value for absorption of NTA after oral administration. However, human data point to a lower absorption rate. A value of 20 % is proposed for risk characterisation purposes.

For 1% NTA dilutions, an absorption rate of 1 % is taken for risk characterisation in humans based on an in vitro dermal absorption study. For higher NTA concentrations in solution and for animals, a higher absorption rate of 10 % is taken for risk characterisation as a default-value based on considerations on the chemical structure and physico-chemical data. Taking into account that oral absorption is 10-20 % which indicates low permeability through biological membranes, a default value of 20 % is proposed for uptake via inhalation.

Acute toxicity

Human data on the acute toxicity of Na₃NTA are not available. Oral LD₅₀ values of about 750 mg/kg bw were obtained for monkeys, of 1300-1470 mg/kg bw for female rats and of 1600-2220 mg/kg bw for male rats. The oral LD₅₀ for dogs exceeds 5000 mg/kg bw. Data on inhalation toxicity are not available. Acute dermal toxicity seems to be low as judged on the basis of a test with rabbits resulting in a minimum lethal dose of more than 10 g/kg bw for rabbits treated with a 25% aqueous substance solution. Based on the available data Na₃NTA has to be classified as „Xn, harmful“ and labelled with „R 22 - harmful if swallowed“.

Irritation / Corrosivity

Human data on local irritancy are not available. Moderate eye irritation was observed in a Draize test, which was not reversible within the study duration (Monsanto Company, unpublished report, 1968). Since the study was terminated on day 7, the reversibility of eye irritation on day 21 could not be assessed as required by existing standards. However, due to the nature of the effects, irreversibility is not expected. Another study indicated recovery from moderate irritation following application of a 38% Na₃NTA solution (BASF, 1982), but this study does not allow to conclude effects of the pure substance. Based on the limited data available and taking also into account the strongly basic pH-value of a 1% aqueous solution, the current classification of R36 - Irritating to eyes - is confirmed.

Sensitisation

A Buehler sensitisation test with Na₃NTA demonstrated a negative result. However, the concentration of 50% used for the induction in this test did not result in any skin irritation. Therefore, this negative result obtained with this Buehler test cannot be considered as valid for the evaluation of the skin sensitizing potential of the substance. The negative data on patch tests with 66 volunteers is not considered to be of sufficient evidence that the substance could not cause contact allergy. The performance of a Local Lymph Node Assay (LLNA, preferred) or a Magnusson Kligman Test could be considered. However, based on a “weight

of evidence approach” based on QSAR considerations and negative findings for 3 structurally related substances (EU “new substances” notifications), further testing may be waived.

Repeated Dose Toxicity

Inhalation exposure

Inhalation studies on rats showed that NTA concentrations of 2 mg/l (6 h/d, 4 d) were irritative to the mucosa of the respiratory tract including the nose and the eyes, whereas at 0.2 mg/l no adverse effects were reported. Prolongation of inhalation exposure to a period of 4 weeks resulted in dyspnoea in rats and guinea pigs at NTA concentration of 0.34 mg/l, the NOAEC for local effects in both species was 0.21 mg/l. No adverse effect on the respiratory system was observed in monkeys at concentrations up to 0.34 mg/l NTA. Systemic effects associated to the inhalation of 0.34 mg/l NTA were diarrhea in monkeys and elevated ASAT activity and protein levels in rats indicating a minor dysfunction of liver cell metabolism. The NOAEC for systemic effects was 0.21 mg/l in rats and monkeys. However, this value is highly uncertain due to the lack of laboratory and pathology examinations.

Rat studies with oral administration

The organ system that was mainly affected by repeated oral treatment with NTA was the urinary tract with lesions at several sites: in the kidneys, ureters and urinary bladder. Histomorphologic kidney lesions were discovered at several segments: the cortex area, the renal papilla and the renal pelvis. The epithelium of the proximal convoluted tubules of the cortex region was found to be primarily affected. Another target tissue was the transitional cell epithelium (urothelium) of the renal pelvis, the ureters and the urinary bladder.

Gross findings after repeated administration were red-brown discoloured urine, enlarged kidneys, increased kidney to body ratio, discoloured kidneys, rough surface of the kidneys, hydronephrosis (pelvic dilation) and dilated ureters. One of the predominant microscopic lesions induced by NTA occurred primarily in the proximal tubules of the cortex region and was consistently reported as vacuolisation (of non-hyperplastic epithelial cells), degeneration of the tubular epithelium, and simple and nodular hyperplasia of the tubules. Tubular hyperplasia was reported to be frequently associated with vacuolisation of tubular cells. Additionally, basophilic (regenerative) hyperplasias without cellular vacuolisation were observed. Erosion/ulceration and hyperplasia of the transitional cell epithelium were observed in the renal pelvis. In studies with sequential sacrifices every fourth day, development of pelvic lesion started later and at a higher dose than tubular damage. After 7 weeks of NTA treatment, transitional cells also appeared to be dysplastic, and showed intracytoplasmic globules, and mitotic figures. Lesions at these two main loci may be associated to secondary responses as a sequelae to the epithelial cytotoxicity. They consisted of inflammation (interstitial or subepithelial), haemorrhage, fibrosis, dilatation of tubules and collection ducts, and tubular mineralisation of these lesions. Ultrastructurally, cellular lesions in the tubules were characterised as swelling, vacuolar degeneration of organelles (endoplasmatic reticulum, mitochondria) and cell lysis.

Renal toxicity was observed in orally treated rats irrespective of the application modus using diet or drinking water containing NTA or gavage administration of NTA. The minimal effective dose that induced toxicity in the urinary tract of rats was 0.15% Na₃NTA (97 mg/kg bw/d) and became evident in this study after treatment with diet on 6 months. LDH enzyme

activities were increased in the urine of rats treated at 20000 ppm (926 mg/kg bw/d) of NTA for 3 weeks. The increase may give indication on a tubular damage.

With respect to the regression of renal lesions, the incidence of basophilic hyperplasia in rats treated for 7 weeks with a diet containing 2% Na₃NTA.H₂O (1309 mg/kg bw/d) and a normal diet until the end of a 5-week recovery period remained increased. However, vacuolar degeneration of tubules and nodular forms of basophilic cell hyperplasia were not longer persistent after the recovery period. Additionally, a higher incidence of hydronephrosis persisted at the end of recovery.

Na₃NTA and H₃NTA provoked in rats inconsistent effects on the urinary volume and pH value. Na₃NTA increased the urine pH values and the volume at doses of 525 mg/kg bw/d or higher. Both parameters were reversely changed after H₃NTA treatment. A dose-related reduction of urine pH-values were also reported for male rats at gavage doses of 150 mg/kg bw/d and higher and female rats at 1500 mg/kg bw/d. Controversially, the urine volume was not changed after 10-weeks of drinking water exposure to concentrations up to 1% Na₃NTA (1000 mg/kg bw/d).

Different findings regarding the NTA-treatment related increase of electrolyte excretion with the urine were observed in the oral studies. No change on the Ca excretion was reported in the BASF study (1998) on rats at the highest dose tested (926 mg/kg bw/d, 4 week). A dose-dependent increase was reported at doses from 1400 mg/kg bw/d Na₃NTA.H₂O in a 3-week rat study thus Ca excretion may only be altered at very high doses. Urinary iron excretion was not altered by Na₃NTA.H₂O treatment for 4 weeks, whereas urinary Zn concentrations were increased in rats at 20000 ppm (926 mg/kg bw/d).

The sediment examination of the urine revealed conflicting results in rats with respect to excretion of crystals. Decreased amounts of crystals were observed in the 4-week study with Na₃NTA.H₂O at a dose of 20000 ppm (926 mg/kg bw/d), but crystalluria was seen after 3- and 4-week administration of doses from 525 mg/kg Na₃NTA.H₂O or H₃NTA. Granular casts and tubular and transitional epithelial cells were observed in rat urine at a gavage dose of 1500 mg/kg bw/d of H₃NTA. Transitional epithelial cells in the urine were transiently seen during a 4-week feeding period of 20000 ppm Na₃NTA.H₂O (926 mg/kg bw/d).

Haematuria and elevated numbers of erythrocytes were seen in urine sediments of treated rats at 20000 ppm (926 mg/kg bw/d) Na₃NTA.H₂O or higher and at 1500 mg/kg bw/d of H₃NTA. Glucosuria indicative for reduced tubular reabsorption was found at 1% Na₃NTA (1000 mg/kg bw/d) given with the drinking water for 10 weeks.

Other effects after oral administration of NTA to rats

Survival rates were reduced in male rats of a 2-year feeding study at a concentration of 0.5% Na₃NTA (322 mg/kg bw/d). Increased mortality rates were also seen in a 10-week drinking water study on rats receiving 1% Na₃NTA (1000 mg/kg bw/d) and in male rats of a 2-year carcinogenicity study at diet concentrations of 20000 ppm NTA (921 mg/kg bw/d), but not at 2000 ppm NTA (92 mg/kg bw/d).

Unspecific toxic effects due to subacute and subchronic administration of NTA were reduced body weight gain, lower feed consumption and feed efficiency at doses of 350 mg/kg bw/d Na₃NTA.H₂O, or 500 mg/kg bw/d H₃NTA and higher. The maximum dose of NTA without abnormalities on the growth was 150 mg/kg bw/d for 3 weeks and 322 mg/kg bw/d Na₃NTA in the first 8 weeks of a 2-year rat study. Diarrhea, smeared anogenital region, perinasal

encrustation and pilorection were observed in rats receiving 1500 mg/kg bw/d by gavage application.

Elevated blood glucose levels in rats were reported after Na₃NTA exposure to concentrations of 0.01% to 1% in drinking water (10-1000 mg/kg bw/d) for 10 weeks, whereas BUN was unchanged. Animals treated at 20000 ppm Na₃NTA.H₂O (2000 mg/kg bw/d) showed reduced RBC counts and hemoglobin content indicating anemia.

Oral Na₃NTA exposure increased Fe concentrations in the rat liver tissue. Although a low percentage of NTA was deposited in the skeleton no clear adverse effect of NTA treatment on the bone was seen in a 2-year feeding study at concentrations of Na₃NTA (322 mg/kg bw/d). Increased deposition of Zn was associated to NTA treatment in rats receiving appr. 239 mg/kg bw/d Na₃NTA within drinking water on 35 days. No indication on relevant alteration of electrolyte homeostasis except higher Zn concentration in bone and Fe concentration in liver was demonstrated in rats that were orally administered to 1 mmol/kg bw/d NTA (191 mg/kg bw/d) for 35 days .

Oral studies in other species

In mice, a 20-week feeding of 1% Na₃NTA.H₂O or H₃NTA induced elevated values of BUN and liver enzyme activities (LDH, ASAT, ALAT). No altered urinary concentrations of Zn, Cu, Mg, Mn, Fe, and Ca were determined in daily urine from mice injected i.p. 100 mg/kg bw/d NTA for 3 consecutive days.

Dogs receiving Na₃NTA on 90 days with feed showed a dose-related increase of NTA content in their bones and a highly variable, but increased urinary Zn excretion (≥ 42.5 mg/kg bw/d), however no changes of hematology and clinical chemistry parameters, metal serum concentrations, urinary and fecal concentrations of Ca, Mg, Fe, and Zn at doses up to 130 mg/kg bw/d. No treatment-related abnormality in gross pathology or histopathology was found in any of 31 organs/tissues (including the kidneys, urinary bladder and bone) at diet concentrations up to 0.5% Na₃NTA (130 mg/kg bw/d).

Oral No/Lowest observed adverse effect level (N/LOAEL)

For the derivation of an overall N/LOAEL all studies were considered that included the examination of toxic effects on the urinary tract. Studies focussing only on very specific questions like the electrolyte status were excluded. The level of N/LOAEL was derived from NTA related toxicity in target organs (mortality increase and renal hydronephrosis at 921 g/kg bw/d). For risk assessment purposes on non-cancer endpoints, a NOAEL of 92 mg/kg bw/d Na₃NTA.H₂O could be proposed from the 24-month cancer study (NCI, 1977). This NOAEL is very close to the lowest dose demonstrated to be carcinogenic - at least in rats at 0.1% (100 mg/kg bw/d) Na₃NTA. Due to the fact that hyperplasias in the urinary tract were the most sensitive effects following long term exposure to NTA, the NOAEL for cytotoxic effects should not be applied for risk assessment on repeated dose toxicity. Instead the BMD on carcinogenicity taking the hyperplasias into account is recommended.

Dermal exposure

A Na₃NTA concentration applied as 2.5% Na₃NTA aqueous solution (2 ml, ≈ 50 mg/kg bw/d) was without irritation thus representing the NOAEL for local effects on the rabbit dermis. No

indication of systemic effects was observed. However, these 28-day and 90-day studies can not be used for derivation of a systemic NOAEL due to incomplete data reporting.

Mutagenicity

Sodium salts of NTA are negative in bacterial test systems for gene mutations. In mammalian cell cultures sodium salts of NTA did not induce gene mutations, sister-chromatid exchanges (SCE), DNA excision-repair (UDS) or DNA strand breaks. NTA seems to have a weak potential for induction of chromosomal aberrations / micronuclei; cell transformation assays were without clear result.

In vivo in mammals, sodium salts of NTA were negative for SCE in bone marrow cells - neither micronuclei and aneuploidy nor SCE. In kidney cells, results were negative for DNA base damage and inconclusive for induction of micronuclei and DNA strand breaks. On the other hand, from in vivo germ cell tests there is limited evidence that i.p. dosing of 275 mg/kg bw has the potential for induction of aneuploidy in mouse spermatocytes. However, positive effects are limited to high doses and a non-linear dose-effect relationship, including a threshold, is to be assumed. Furthermore, these findings suffer from methodological insufficiencies. Screening assays for dominant lethal mutations were negative. From the whole amount of data on mammalian somatic cells in vitro and in vivo, there is no plausible evidence for in vivo mutagenicity of NTA and its sodium salts.

Carcinogenicity

In rats and mice, long-term treatment with NTA was associated with tumor development in the urinary tract. Oral 2-year studies in rats revealed tubular cell neoplasms and transitional cell neoplasms in the kidneys, and transitional cell neoplasms in the ureters and urinary bladder. In the mouse, the prominent types of tumors originated from the renal tubular cell. The minimum effective concentration that caused increased incidences of tumors in the urinary tract with origin from the tubular cell epithelium was 0.1% NTA within the drinking water (100 mg/kg/d) (Goyer et al., 1981). The NTA-related increase in tumors with origin from the transitional cell epithelium started at concentrations of 7500 ppm NTA with diet corresponding to 355 mg/kg bw/d Na₃NTA in rats (NCI, 1977). Comparing tumor response between species, the mouse was less sensitive than the rat where NTA-associated tumors occurred at lower dose, at more tumor sites and with a higher variability in tumor spectrum.

In rats, numerous adenomas and carcinomas of the transitional cell epithelium were observed in the renal pelvis (8%), the ureters (29%) and the urinary bladder (13%) of animals (males & females) that received diet NTA concentrations of 20000 ppm (921 mg/kg bw/d) for 24 months (NCI, 1977). In this study, a single papilloma of the urinary bladder seen at 2000 ppm (92 mg/kg bw/d) might also be considered as treatment-related, because of the absence of urinary tract tumors in the study controls, their very low spontaneous incidences in rats and a high rate of urinary bladder tumors at the high dose.

Preneoplastic lesions such as transitional cell hyperplasia and dysplasia of the renal pelvis, the ureter, and the urinary bladder occurred at all dose levels tested including the lowest tested of 200 ppm (9 mg/kg bw/d). No tumors at these locations have been observed in control animals of both studies. While the increase in incidence of hyperplasias and dysplasias were low at 200 ppm and this dose can be used as the non-cancer effect level, the number of hyperplasias markedly increased at 2000 ppm (92 mg/kg/d) (NCI, 1977). Not cytotoxicity (overall NOAEL

92 mg/kg/d) but hyperplasia of the transitional cell epithelium at the same dose represents the most sensitive effect underlying the tumor development.

A benchmark dose was calculated using the U.S.EPA BMD software (Version 1.3.2). Data fitted best when the Gamma Multi-Hit model was applied (AIC criterion 46.7). By this model the benchmark dose lower bound (BMDL) where a treatment-related increase in response of 10% could not be expected within the 95% confidence interval on the BMD was 5.7 mg/kg bw/day. It is proposed to use this value as starting point for quantitative risk characterisation.

Increased incidences in transitional cell epithelium tumors of the ureter (3%) and the urinary bladder (12%) were also seen after administration of diet concentrations of 15000 ppm NTA (1053 mg/kg bw/d) during 18 months followed by a 6-month recovery period (NCI, 1977).

Renal tubular cell neoplasms resulted from NTA administration with the drinking water at a concentration of 0.1 % (100 mg/kg/d) and within diet at a concentration of 20000 ppm (921 mg/kg bw/d). Increased incidence or severity grades of tubular cell hyperplasias considered as preneoplastic lesions were reported for both studies. Tumor incidences of total numbers of tubular cell neoplasms were 15.8% (controls 2.7%) in the Goyer et al. study and 16.6% (controls 0%) in the NCI study.

In mice, kidney tumors in male and female mice have been observed at NTA doses of 7500 and 15000 ppm (725 and 1500 mg/kg/d) after 18 months consumption with feed followed by 6 months of recovery. The kidney was the only tumor site, where numerous tubular cell neoplasms (6% and 29% at low and high dose, respectively) and a single tumor at the renal pelvis occurred. No tubular cell hyperplasia were seen in treated animals, and single transitional epithelium hyperplasias were seen in the renal pelvis and ureter of one low and one high dose mouse.

The medium-term initiation-promotion studies on the urinary bladder as well as on the kidney effects on rats and mice succeeded to demonstrate the acceleration of the tumor development initiated by nitrosamines. These findings, however, do not necessarily indicate that NTA exclusively shows tumor-promoting activities. Beyond the promotive action demonstrated, these studies were able to show that $\text{Na}_3\text{NTA}\cdot\text{H}_2\text{O}$ and H_3NTA (without initiation) induced high numbers of hyperplasias in the urinary bladder and the kidneys after relatively short time periods (28-30 weeks) related to the rat life time. As tubular hyperplasias were frequently observed in tumor bearing animals of conventional carcinogenicity studies, they are considered to represent interim stages in the development of renal tumors which originate from the tubular epithelial cells. The hyperplasias observed in the non-initiated NTA-treated groups of the initiation-promotion studies give support on the view of a genuine carcinogenic potential of NTA.

A drinking water study with Na_2NTA on the development of lung tumors was negative after 26 weeks of treatment followed by a 10-week period of recovery. Here, treatment period might have been too short. A mouse cancer study with subcutaneous injection of NTA did not reveal higher rates of skin tumors.

In one of the 18-months studies with NTA (NCI, 1977), there were higher tumor incidences in the livers, lungs and the adrenals of animals which were treated with NTA compared to those of the control groups. As the control animals presented a relatively high rate of spontaneous tumors at these organs, and equivalent findings were not reported by other studies, it was considered that their association to the treatment is inconclusive.

Reproductive toxicity

There are no human data available for reproductive toxicity of NTA. Data from older investigations in laboratory animals on reproduction and development are not sufficiently detailed reported. From the overall information so far available, any specific teratogenic potential of NTA is not indicated, although transplacental passage and accumulation in the fetal skeleton had been demonstrated. From the results of a combined reproduction and teratology study it appears, that NTA at least up to doses of 250 mg/kg bw/d did not interfere with embryo/fetal development in rabbits, while in rats NTA dose levels of at least approximately 450 mg/kg bw/d did not adversely affect reproductive performance and/or embryo/fetal development.

4.1.3.2 Workers

4.1.3.2.1 Introductory remarks

For occupational risk assessment of trisodium nitrilotriacetate the MOS approach as outlined in the revised TGD is applied. This occupational risk assessment is based upon the toxicological profile of trisodium nitrilotriacetate (chapter 4.1.2) and the occupational exposure assessment (chapter 4.1.1.2). The threshold levels identified in the hazard assessment are taken forward to this occupational risk assessment.

This introductory remark specifies the route-specific information on absorption, applies these absorption data to transform the external occupational exposure levels to the corresponding internal body burden, and gives a short introduction to the MOS approach used.

Systemic availability for different routes of exposure

For the majority of toxicological endpoints trisodium nitrilotriacetate data originate from oral studies. Since workers are exposed either by inhalation or by skin contact, route-to-route transformation is essential for the occupational risk assessment.

Experimental data with rats show a median value for oral absorption of about 50%. With reference to the chapter on toxicokinetics (see under 4.1.2.1) a value of 100% for oral absorption in rats is discussed. However, a value of 50% for oral absorption in the rat is taken, sticking on the agreement of the last member state discussion (TCNES II '07), knowing, that this approach leads to lower critical exposure levels and thus is more conservative. 20% is taken for the oral absorption of humans.

There are no data known concerning absorption after inhalation. Considerations about the chemical structure and physico-chemical data (molecular weight, water solubility, partition coefficient and ionisation state) result in a default value 20% absorption after inhalation.

Experimental in vitro data show very low absorption percentages after application of radiolabeled NTA on human skin (see under 4.1.2.1). At the low dose dilution (1% NTA), absorption percentages ranged between 0.042 and 0.472 % within the five different samples. For higher NTA concentrations in solution, a higher absorption rate of 10 % (default based on physico-chemical properties due to the lack of meaningful experimental data) is taken for risk

characterization, knowing that this very conservative approach might take the human variability adequately into account.

In table 4.1.3.2.A the exposure levels of table 4.1.1.2.2 are summarised and the route-specific and total internal body burdens are identified.

Table 4.1.3.2.1.A: Trisodium nitrilotriacetate exposure levels which are relevant for occupational risk assessment and internal body burden.

Exposure scenario			Inhalation		Dermal contact		Internal body burden		
							Inhalation ⁽¹⁾	Dermal ⁽²⁾	Combined
			mg/m ³	mg/kg/d	mg/p/d	mg/kg/d	mg/kg/d		
1a	Production of Na ₃ NTA	dust	3.9 ⁽³⁾	0.56	42 ⁽⁴⁾	0.6	0.11	0.06	0.17
1b		liquid			8.4 ⁽⁴⁾				
2a	Use of Na ₃ NTA in formulation process (up to 40%)	dust	0.62 ⁽⁷⁾	0.09	3,000 ⁽⁶⁾	42.8	0.02	4.28	4.3
			6.25 ⁽⁸⁾	0.89					0.18
2b		liquid	negl.	negl.	4,600 ⁽⁶⁾	65.7	negl.	6.57	6.6
3a		High pressure cleaning (diluted solutions, < 2% NTA)	droplet aerosols	0.3 ⁽⁶⁾	0.04	252 ⁽⁵⁾	3.6	0.01	0.36
3b	liquid		19 ⁽⁵⁾			0.27			

⁽¹⁾based on the assumption of 20% inhalative absorption; breathing volume of 10 m³ per shift

⁽²⁾based on the assumption of 10% systemic availability of trisodium nitrilotriacetate after dermal contact

⁽³⁾measurement data

⁽⁴⁾EASE-estimation (90% protection by suitable gloves)

⁽⁵⁾EASE-estimation without gloves

⁽⁶⁾determined by analogy (calculation without gloves)

⁽⁷⁾EASE with LEV

⁽⁸⁾EASE without LEV

MOS Approach

The MOS approach for human risk characterisation is described in detail in chapter 4 of the revised TGD. The following chapter contains a short introduction to the MOS approach used. The basic principle of the MOS approach is a comparison of scenario-specific MOS values (the relationship between the experimental NOAEL respectively the adjusted starting point and the exposure level) with a reference MOS (product of various assessment factors).

MOS calculation and the adequate starting point

Basically, MOS values are calculated as quotient of a relevant NOAEL from experimental animal testing or human studies and actual workplace exposure levels. In specific situations, the MOS approach requires converting the original NOAEL into an adequate starting point or

corrected NOAEL previously to MOS calculation in order to be directly comparable to the exposure assessment. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental data should be converted to the dose unit of the exposure data.

For occupational risk assessment, the corrected inhalatory NOAEC accounts for the difference of the standard respiratory volume (6.7 m³) and the respiratory volume for light activity (10 m³).

MOS values are calculated for different routes of exposure and for different toxicological endpoints. The routes of exposure specifically considered in occupational risk assessment are inhalation exposure and dermal contact.

In addition, for risk assessment of combined exposure (inhalation exposure and dermal contact) an adequate internal NOAEL is derived from external NOAELs and specific information on route-specific absorption. For MOS calculation, the adjusted internal starting point is divided by the internal body burden. Depending on route-specific exposure and absorption, inhalation exposure and/or dermal exposure may contribute to the internal body burden. With respect to the possible outcome of an assessment for combined risks, interest focuses on scenarios with conclusion ii at both exposure routes. Based on theoretical considerations, combined exposure will not increase the most critical route-specific risk component more than twice.

Reference MOS

The MOS values calculated have to be compared with a reference MOS. The reference MOS is an overall assessment factor, which is obtained by multiplication of individual assessment factors. The Technical Guidance Document emphasises several aspects which are involved in the extrapolation of experimental data to the human situation. For these assessment factors, default values are recommended. It is important to point out that any relevant substance-specific data and information may overrule the defined default values.

Interspecies extrapolation on the one hand is based on allometric scaling. For remaining interspecies differences the revised TGD proposes an additional factor of 2.5.

For workers, an adjustment factor for intraspecies differences of 5 is recommended. Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%).

For chemical substances it is usually expected that the experimental NOAEL will decrease with increasing duration of application. Furthermore, other and more serious adverse effects may appear with prolonged exposure duration. For duration adjustment, a default factor of 6 is proposed for extrapolation from a subacute to chronic exposure. The duration adjustment factor is lower (a factor of 2) for the transition from subchronic experimental exposure to chronic exposure.

The TGD defines two further adjustment factors (uncertainty in route-to-route extrapolation and dose-response relationship including severity of effect). In specific cases these factors may be different from one.

Adjustment factors for trisodium nitrilotriacetate

For trisodium nitrilotriacetate the oral NOAELs (usually from rat studies) were transformed into the adequate starting points for dermal contact and inhalation exposure. Because of experimental data oral absorption of the rat is assumed to be 50%. By default a 10% dermal and 20% inhalative absorption has been taken. For occupational risk assessment, the corrected inhalatory NOAEC accounts for the difference of the standard respiratory volume (6.7 m³) and the respiratory volume for light activity (10 m³).

For interspecies extrapolation for the rat the allometric scaling factor of 4 and the factor of 2.5 for remaining differences are applied. For intraspecies extrapolation, the default factor of 5 was used.

Because relevant long-term studies are available, for systemic effects no duration adjustment was necessary. With respect to local effects a reduced duration adjustment factor of 2 was taken, because no significant change of the NOAEC from a short term study compared with a 4 week study was reported.

Comparison of MOS and reference MOS

The MOS values for different toxicological endpoints and different exposure scenarios are compared with the substance- and endpoint-specific reference MOS. MOS values clearly above the reference MOS do not lead to concern, whereas MOS values that are clearly below the reference MOS are cause for concern. There may be various risk-related aspects which are not covered by default assessment factors. These additional qualitative aspects should be carefully considered when performing a risk assessment and should have adequate influence on finding of conclusions.

Critical Exposure Levels

In a parallel procedure, which gives identical but more direct results, the adjusted toxicological starting point is directly divided by the reference MOS. As a result, an exposure level (in mg/m³ or mg/kg/d) is identified, which may serve as a direct trigger for decisions when compared with the occupational exposure levels. In the context of this risk assessment report this trigger value is called "critical exposure level". Concern will be expressed for scenarios with occupational exposure levels higher than the relevant "critical exposure level".

4.1.3.2.2 Occupational Risk Assessment

Acute toxicity

The lowest oral LD₅₀ values of 1,300-1,470 mg/kg are described for female rats. From a reprotoxicity study at rats a NOAEL of 450 mg/kg/day is reported. The minimum dermal lethal dose of trisodium nitrilotriacetate for rabbits is more than 10,000 mg/kg.

A repeated inhalation study from EPA (1980) on rats and guinea pigs showed no adverse effects at an exposure level of 210 mg/m³ trisodium nitrilotriacetate for a time period of 4 weeks (no histopathology was done).

To assess the acute risks after trisodium nitrilotriacetate exposure the NOAEL from the reprotoxicity study is taken (450 mg/kg/day), taking also into account that compared to the NOAEL for repeated dose toxicity (see below) this NOAEL is plausible and careful enough.

Internal starting point

The calculation of the internal starting point has to account for a factor of 1/2 for 50% oral absorption for the rat. This gives an internal value of 225 mg/kg/day ($450 \times \frac{1}{2}$).

Inhalative exposure

Assuming 20% absorption by inhalation, the internal starting point has to be multiplied with 5 to get the external inhalation dose. This results in a value of 1,125 mg/kg/day ($225 \text{ mg/kg/day} \times 5$). The inhalation dose of 1,125 mg/kg/day is divided by a factor of 0.38 m³/kg (rat breathing volume during 8 hours) and is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an inhalative starting point of 1,985 mg/m³ ($1,125 \times \frac{1}{0.38} \times \frac{6.7}{10}$).

For the identification of the reference MOS the interspecies factor of 2.5 for remaining differences is multiplied with an intraspecies factor of 5 which results in a reference MOS of 12.5 (2.5×5). The corresponding critical exposure level calculates to 159 mg/m³ ($1,985 / 12.5$).

The highest inhalative exposure value of 6.25 mg/m³ results from scenario 2a (use of Na₃NTA in formulation process, without LEV). Comparing this value with the critical exposure level of 159 mg/m³ there is no reason for concern (see table 4.1.3.2.B).

Conclusion: ii

Dermal exposure

The internal starting point of 225 mg/kg/day is multiplied by the factor of 10 to account for 10% dermal absorption. This results in a value of 2,250 mg/kg/day (225×10) for the adequate dermal starting point.

For the calculation of the reference MOS an interspecies factor of 4 x 2.5 (rat) is taken. For intraspecies differences no further factor is used, because the dermal absorption percentage of 10%, taken from the experimental data (see chapter 4.1.2.1) is a very conservative approach which took the human variability into account. This results in a reference MOS of 10 (4×2.5) the corresponding critical exposure level calculates to 225 mg/kg/day ($2250 / 10$). There is no concern with respect to dermal acute toxicity (see table 4.1.3.2.B).

Conclusion: ii

Combined exposure

The internal starting point of 225 mg/kg/day is divided through the reference MOS of 10 (the reference MOS for internal and dermal exposure is identical). The corresponding critical exposure level calculates to 22.5 mg/kg/day ($225 / 10$).

There is no concern with respect to acute toxicity after combined exposure of trisodium nitrilotriacetate.

Conclusion: ii

Table 4.1.3.2.2.B: Estimation of MOS values for acute toxicity of trisodium nitrilotriacetate

			Inhalation			Dermal			Combined		
Starting point for MOS calculation			1,985 mg/m ³			2,250 mg/kg/day			225 mg/kg/day		
Reference MOS			12.5			10			10		
Critical exposure level			159 mg/m ³			225 mg/kg/day (external)			22.5 mg/kg/day (internal)		
			Exposure (mg/m ³)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1a	Production of Na ₃ NTA	dust	3.9	509	ii	0.6	3,750	ii	0.17	1,324	ii
1b		liquid									
2a	Use of Na ₃ NTA in formulation process (up to 40%)	Dust, with LEV	0.62	3,200	ii	42.8	53	ii	4.3	52	ii
		Dust, without LEV	6.25	318	ii				4.46	50	ii
2b		liquid	negl.	-	ii	65.7	34	ii	6.6	34	ii
3a	High pressure cleaning (diluted solutions, < 2% NTA)	droplet aerosols	0.3	6,620	ii	3.6	625	ii	0.37	608	ii
3b		liquid				0.27	8,330	ii	0.04	5,625	ii

Irritation/Corrosivity

Skin

There are no human data on skin irritation. Experimental data (Draize test, rabbit, 25%-solution, 24-hour exposure) only indicate mild acute skin irritation. Trisodium nitrilotriacetate is not considered to be a skin irritating substance.

Conclusion: ii

Eyes

Moderate eye irritation was observed in a Draize test, which was not reversible within the study duration (Monsanto Company, unpublished report, 1968). Since the study was terminated on day 7, the reversibility of eye irritation on day 21 could not be assessed as required by standard protocols. However, due to the nature of the effects, irreversibility is not expected. Another study indicated recovery from moderate irritation following application of a 38% trisodium nitrilotriacetate solution (BASF, 1982), but this study does not allow to conclude effects of the pure substance. Based on the limited data available, the current classification of R36 - Irritating to eyes - is confirmed.

For those trisodium nitrilotriacetate preparations which are classified and labelled as irritating to the eyes, conclusion ii is proposed on the grounds that control measures exist which can minimise exposure and risk of irritation, thereby reducing concern. However, these controls must be implemented and complied with to reduce the risk of irritation to the eyes.

Conclusion: ii

Inhalative irritation

There is no evidence for respiratory tract irritation in humans. In a rat inhalation study (6 h/d, 4 d) excessive concentrations of 2,000 mg/m³ trisodium nitrilotriacetate were irritative to the mucosa of the respiratory tract including the nose and the eyes. At 200 mg/m³ no adverse effects were reported (EPA, 1980)., With reference to the highest occupational exposure levels of about 5 mg/m³ (shift average), a concern for acute respiratory tract irritation cannot be recognized, since significant reactions only occurred at highest concentrations .

Conclusion: ii

Sensitisation

Skin

Available data on skin sensitisation (Buehler test, human patch tests) do not show a skin sensitising potential, but these data are not considered sufficiently reliable in order to properly assess the skin sensitising potential of trisodium nitrilotriacetate. The performance of a Local Lymph Node Assay (LLNA, preferred) or a Magnusson Kligman Test could be considered. However, based on a "weight of evidence approach" based on QSAR considerations and negative findings for 3 structurally related substances (EU "new substances" notifications), further testing may be waived Overall, it can be concluded that there is no evidence to conclude a skin sensitising potential for NTA and additional testing may be waived.

Conclusion: ii

Respiratory sensitisation

No information on the sensitising potential of the substance at the respiratory tract is available. For the time being a valid study to investigate respiratory sensitisation in experimental animals cannot be recommended. However, trisodium nitrilotriacetate is not suspected to be a potent respiratory sensitiser in humans according to the fact that during all

the years of use no notice of specific case reports has been given. There is no indication of concern with respect to respiratory sensitisation at the workplace.

Conclusion: ii

Repeated dose toxicity

Inhalation (local effects)

Inhalation studies on rats showed that excessive trisodium nitrilotriacetate concentrations of 2,000 mg/m³ (6 h/d, 4 d) were irritative to the mucosa of the respiratory tract including the nose and the eyes, whereas at 200 mg/m³ no adverse effects were reported. Prolongation of inhalation exposure to a period of 4 weeks resulted in dyspnoe in rats and guinea pigs at trisodium nitrilotriacetate concentration of 340 mg/m³, the corresponding NOAEC for local effects in both species was 210 mg/m³. Histopathology data were absent, (see chapter 4.1.2.6). The experimental NOAEC of 210 mg/m³ is taken to assess occupational risks after repeated inhalation.

The NOAEC is multiplied by a factor of 6.7/10 (activity-driven differences of respiratory volumes in workers) and a factor of 6/8 (differences between experimental exposure duration and occupational shift length of 8 h). This results in a value of 105 mg/m³ as inhalation starting point (210 mg/m³ x 6.7/10 x 6/8).

For the identification of the reference MOS the interspecies factor of 2.5 for remaining differences is multiplied with an intraspecies factor of 5. A reduced default factor to adjust for possible differences of thresholds for short-term and chronic exposure of 2 is used, because no difference between the NOAECs of the short-term study and the 4 week study was reported.

This gives altogether a reference MOS of 25 (2.5 x 5 x 2). The critical exposure level regarding local effects after repeated exposure is identified as 4.2 mg/m³ (105 / 25).

Concern results for scenario 2a: Use of Na₃NTA in formulation process (up to 40%, dust, without LEV). The other scenarios are out of concern (see table 4.1.3.2.C).

Conclusion: iii

Table 4.1.3.2.2.C: MOS values for local effects of trisodium nitrilotriacetate after repeated inhalation

Starting point for MOS calculation		105 mg/m ³		
Reference MOS		25		
Critical exposure level		4.2 mg/m ³		
		Exposure (mg/m ³)	MOS	Conclusions
1. Production of Na ₃ NTA		3.9	27	ii
2a Use of Na ₃ NTA in formulation process (up to 40%), dust	with LEV	0.62	170	ii
	without LEV	6.25	17	iii
2b Use of Na ₃ NTA in formulation process (up to 40%), liquid		negl.	-	ii
3. High pressure cleaning (diluted solutions, < 2% NTA)		0.3	350	ii

Dermal contact (local effects)

Two ml/kg/day of a 2.5% aqueous solution of trisodium nitrilotriacetate (≈ 50 mg/kg/day) was applied to the clipped and abraded backs (no data on cm² of skin area) of 6 rabbits for a period of 91 days (65 treatments) (Nixon, 1971). No adverse effects were observed. However, the data reported were incompliant to standard test design.

Since in a Draize test with rabbits a 25% trisodium nitrilotriacetate-solution resulted in mild acute skin irritation after 24 hours, it cannot be excluded that repeated exposure to formulations with more than 25% trisodium nitrilotriacetate might result in slight irritation to the skin. Risk reduction is necessary, because after repeated exposure of trisodium nitrilotriacetate systemic effects are described and extrapolation leads to a low critical exposure level (see below). Therefore no further repeated dose toxicity study, regarding the dermal local effects is warranted.

Conclusion: ii

Systemic effects (RDT) by inhalation, dermal contact, combined exposure

No human data are available. Animal studies after repeated oral intake of trisodium nitrilotriacetate show, that the mainly affected organ system is the urinary tract with lesions at several organs: the kidneys, ureters and urinary bladder. Target tissues are the epithelium of the proximal convoluted tubels of the cortex region and the transitional cell epithelium of the renal pelvis, the ureters and the urinary bladder.

For the assessment of repeated dose toxicity an oral 24-month carcinogenicity rat study with trisodium nitrilotriacetate is used (NCI, 1977). In this study trisodium nitrilotriacetate was tested at diet concentrations of 0, 200, 2,000, and 20,000 ppm (appr. 0, 9, 92, 921 mg nitrilotriacetate/kg/day, for detailed description see chapter 4.1.2.8). Increased numbers of dysplasias in the urinary bladder and transitional cell hyperplasias were seen in the renal pelvis, the ureter and in the urinary bladder of males and females at all dose groups (see table 4.1.2.8.1). At the high dose level body weight was significantly reduced and the mean survival rate for males was significantly lower. Additionally hydronephrosis was evident at the high dose level in most of the animals. Based on the findings of mortality increase and renal hydronephrosis at the high dose level, a NOAEL for toxic lesions could be estimated to 2,000 ppm (92 mg/kg/day). However, at this dose preneoplastic lesions in rats were increased since hyperplasias were observed at lower doses than cytotoxicity. The hyperplasias are interpreted as the relevant toxic effect and serve as basis for the assessment of repeated dose toxicity.

It is preferred to use a BMD value, calculated for the increase of hyperplasias. The BMDL10 regarding the hyperplasias was 5.7 mg/kg/day, when the Gamma Multi-Hit model was applied. This value serves as basis for the risk assessment of repeated dose toxicity (and later on for carcinogenicity).

Internal starting point

The calculation of the internal starting point has to account for a factor of 1/2 for 50% oral absorption for the rat, resulting from experimental data (see under 4.1.2.1). The BMDL of 5.7 mg/kg/day will be transformed to an internal starting point of 2.85 mg/kg/day ($5.7 \times \frac{1}{2}$).

Inhalative exposure

Assuming a 20% absorption by inhalation, the internal starting point has to be multiplied by 5 to get the inhalatory dose. This results in a value of 14.3 mg/kg/day ($2.85 \text{ mg/kg/day} \times 5$). The inhalatory dose of 14.3 mg/kg/day is divided by a factor of 0.38 m³/kg (rat breathing volume during 8 hours) and is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an inhalative starting point of 25 mg/m³ ($14.3 \times \frac{1}{0.38} \times \frac{6.7}{10}$).

For the identification of the reference MOS the interspecies factor of 2.5 for remaining differences is multiplied with an intraspecies factor of 5. This gives altogether a reference MOS of 12.5 (2.5×5). The corresponding critical exposure level calculates to 2 mg/m³ ($25 / 12.5$).

Based on this calculation, scenario 1 (Production of Na₃NTA) and scenario 2a (use of Na₃NTA in formulation process, dust without LEV) reach concern (see table 4.1.3.2.D).

Conclusion: iii

Dermal exposure

The internal starting point of 2.85 mg/kg/day is multiplied by the factor of 10 to account for a 10% dermal absorption. This results in a value of 28.5 mg/kg/day (2.85×10) for the adequate dermal starting point.

For the calculation of the reference MOS an interspecies factor of 4 x 2.5 (rat) is taken. For intraspecies differences no further factor is used, because the dermal absorption percentage of 10%, taken from the experimental data is a very conservative approach which took the human variability into account. This results in a reference MOS of 10 (4 x 2.5) the corresponding critical exposure level calculates to 2.85 mg/kg/day (28.5 / 10).

Conclusion iii is expressed for scenarios 2a and 2b (use of Na₃NTA in formulation process, dust resp. liquid) and scenario 3a (high pressure cleaning with droplet aerosols). Particularly the concern for scenario 2a and 2b is pronounced, because the critical exposure level is exceeded by a factor of 15-25 (42.8 and 65.7 mg/kg/day exposure versus the critical exposure level of 2.85 mg/kg/day, see table 4.1.3.2.D).

Conclusion: iii

Combined exposure

The internal starting point of 2.85 mg/kg/day is divided through the reference MOS of 10 (the reference MOS for internal and dermal exposure is identical). The corresponding critical exposure level calculates to 0.285 mg/kg/day (2.85 / 10).

Nearly all scenarios reach concern except scenario 3b (high pressure cleaning, diluted solutions, < 2% NTA of liquids). This conclusion already results at least from a single route of exposure (inhalation and/or dermal), therefore no scenario has a specific concern regarding combined exposure.

Conclusion: iii

Mutagenicity

From the whole amount of data on mammalian somatic cells in vitro and in vivo, there is no plausible evidence for in vivo mutagenicity of NTA and its sodium salts. There is no reason for concern.

Conclusion: ii

Carcinogenicity

Carcinogenicity of inhalation, dermal, and combined exposure

There are several oral studies with administration of trisodium nitrilotriacetate via the diet or the drinking water available. These studies showed that long-term treatment with trisodium nitrilotriacetate was associated with tumor development in the urinary tract. Occupational risk assessment will rely on the results of the 24 months-study in rats with application of trisodium nitrilotriacetate in the diet. The study was also taken for the risk assessment of repeated dose toxicity (see above). F344 rats in groups of 24 males and 24 females were feeded with diet concentrations of 0, 200, 2,000, and 20,000 ppm trisodium nitrilotriacetate (appr. 0, 9, 92, 921 mg/kg/day). At all doses increased numbers of transitional cell hyperplasia of the urinary tract were seen with a significant increase at the middle and high dose group (see table 4.1.2.8.1). Additionally one tumor (papilloma of the bladder) was found in a mid dose female, and

several tumors at different stages were seen at multiple localisations (kidney, ureter, urinary bladder) at the high dose level.

Based on the actual knowledge a direct genotoxic mechanism of trisodium nitrilotriacetate carcinogenesis could not be demonstrated. For carcinogenic risk assessment it is assumed that carcinogenicity is mediated by a non-genotoxic (epigenetic) mechanism.

Based on the available data (see chapter on hazard assessment) the substance-induced development of hyperplasia (in the urinary bladder of the female rat) is taken as a primary adverse effect, finally resulting in the development of tumors. Thus the risk assessment for carcinogenicity starts with the same BMDL10 value of 5.7 mg/kg/day which was used for the assessment of repeated dose toxicity. The formal assessment factors and conclusions on carcinogenicity are therefore identical to the values for repeated dose toxicity. The calculations are shown in the chapter above and in table 4.1.3.2.D.

There is need for risk reduction after inhalation exposure for scenario 1 (Production of Na₃NTA) and scenario 2b (use of Na₃NTA in formulation process without LEV), and for dermal exposure of the scenarios 2a and 2b (use of Na₃NTA in formulation process, dust resp. liquid) and scenario 3a (high pressure cleaning with droplet aerosols). Especially the dermal exposure resulting from scenarios 2a and 2b has to be reduced, because exposure exceeds by a factor of about 40-60 compared with the critical exposure level.

Conclusion: iii

Table 4.1.3.2.2.D: MOS values for RDT and cancer risks by trisodium nitrilotriacetate

			Inhalation			Dermal			Combined		
Starting point for MOS calculation			25 mg/m ³			28.5 mg/kg/day			2.85 mg/kg/day		
Reference MOS			12.5			10			10		
Critical exposure level			2 mg/m ³			2.85 mg/kg/day (external)			0.285 mg/kg/day (internal)		
			Exposure (mg/m ³)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1a	Production of Na ₃ NTA	dust	3.9	6.4	iii	0.6	48	ii	0.17	17	iii ⁽¹⁾
1b		liquid				0.12	238	ii	0.12	24	iii ⁽¹⁾
2a	Use of Na ₃ NTA in formulation process (up to 40%)	Dust, with LEV	0.62	40	ii	42.8	0.7	iii	4.3	0.6	iii ⁽¹⁾
		Dust, without LEV	6.25	4	iii				4.46	0.6	iii ⁽¹⁾
2b		liquid	negl.	-	ii	65.7	0.4	iii	6.6	0.4	iii ⁽¹⁾
3a	High pressure cleaning (diluted solutions, < 2% NTA)	droplet aerosols	0.3	83	ii	3.6	8	iii	0.37	7.7	iii ⁽¹⁾
3b		liquid				0.27	148	ii	0.04	71	ii

⁽¹⁾conclusion iii already results from inhalative and/or dermal exposure, therefore no specific concern regarding combined exposure is expressed

Reproductive toxicity

Fertility impairment and developmental effects by inhalation, dermal and combined exposure

Trisodium nitrilotriacetate did not adversely affect reproductive performance and capability through two successive generations in rats at dietary levels of approximately 450 mg/kg/day, whereas some mild toxicity (not further specified) had been reported. In addition, any specific teratogenic potential and/or impairment of embryo/fetal development are not indicated from the same data. Therefore no MOS calculation is performed for this endpoint. There is no reason for concern.

Conclusion: ii

Summary for occupational risk assessment of trisodium nitrilotriacetate

Table 4.1.3.2.2.E indicates the toxicological endpoints of concern for trisodium nitrilotriacetate. The most critical endpoints are repeated dose toxicity and carcinogenicity after inhalation and dermal contact and local effects after repeated inhalation.

Table 4.1.3.2.2.E: Endpoint-specific overall conclusions

Toxicological endpoints		General conclusion	Exposure Scenarios
Acute toxicity	inhalation	ii	
	dermal	ii	
	combined	ii	
Irritation/ Corrosivity	dermal	ii	
	eye	ii	
	acute respiratory tract	ii	
Sensitisation	skin	ii	
	respiratory	ii	
Repeated dose toxicity	inhalation, local	iii	2a
	inhalation, systemic	iii	1, 2a ⁽²⁾
	dermal, local	ii	
	dermal, systemic	iii	2, 3a
	combined, systemic	iii	1, 2, 3a
Mutagenicity		ii	
Carcinogenicity	inhalation	iii	1, 2a
	dermal	iii	2, 3a
	combined	iii	1 ⁽¹⁾ , 2 ⁽¹⁾ , 3a ⁽¹⁾
Reproductive toxicity	inhalation	ii	
	dermal	ii	
	combined	ii	

⁽¹⁾conclusion iii already results from inhalative and/or dermal exposure, therefore no specific concern for the combined exposure scenario is indicated

⁽²⁾without LEV

Risk estimation is mainly based on oral studies. There are no data known concerning absorption after inhalation or dermal application. Because of the chemical structure and physico-chemical data (see chapter 4.1.2.1.) 20% absorption is assumed as default value for absorption via the inhalative exposure, and 10% for dermal absorption. These values were taken forward to risk characterisation.

The most critical toxicological endpoint is the systemic toxicity after repeated exposure respective carcinogenicity. For the risk assessment the increase of hyperplasias served as combined starting point of both endpoints, assuming that carcinogenicity is mediated by a non-genotoxic (thresholded) mechanism. The collective starting point was calculated by using a Benchmark dose computation. The formal assessment factors and conclusions on carcinogenicity were identical for both endpoints. Thus an identical critical exposure level for both endpoints was identified.

Besides scenario 3b (high pressure cleaning (diluted solutions, <2% NTA) for liquids), all scenarios reach concern. Especially dermal exposure (with a critical exposure level of 2.85 mg/kg/day) has to be reduced for scenario 2 and 3, and inhalation exposure (with a critical exposure level of 2 mg/m³) has to be reduced for scenario 1 and 2b.

Tables 4.1.3.2.2F (inhalation) and 4.1.3.2.2G (dermal contact) try to visualize the risk profile of trisodium nitrilotriacetate. According to the specific arrangement of exposure scenarios you will find the relatively high risks at the left upper corner, the relatively low risks at the bottom of the right corner. This table may help to reach consistent conclusions for different endpoints and scenarios.

Table 4.1.3.2.2F: Ranking of occupational risks (inhalation) for trisodium nitrilotriacetate

Exposure scenario	Exposure (mg/m ³)	Carcinogenicity	Repeated dose toxicity (systemic)	Repeated dose toxicity (local)	Acute toxicity
		Critical exposure level in mg/m ³			
		2 mg/m ³	2 mg/m ³	4.2 mg/m ³	159 mg/m ³
a. Use of Na ₃ NTA in formulation process (up to 40%), dust without LEV	6.25	iii	iii	iii	ii
1. Production of NTA	3.9	iii	iii	ii	ii
2a. Use of Na ₃ NTA in formulation process (up to 40%), dust with LEV	0.62	ii	ii	ii	ii
3. High pressure cleaning (diluted solutions, <2% NTA)	0.3	ii	ii	ii	ii
2b. Use of Na ₃ NTA in formulation process, liquid (up to 40%)	negl.	ii	ii	ii	ii

Table 4.1.3.2G: Ranking of occupational risks (dermal contact) for trisodium nitrilotriacetate

Exposure scenario	Exposure (mg/kg/d)	Carcinogenicity	Repeated dose toxicity (systemic)	Acute toxicity
		Critical exposure level in mg/kg/d		
		2.85	2.85	225
2b.Use of Na ₃ NTA in formulation process (up to 40%), liquid	65.7	iii	iii	ii
2a.Use of Na ₃ NTA in formulation process (up to 40%), dust	42.8	iii	iii	ii
3a.High pressure cleaning (diluted solutions, <2% NTA), droplet aerosols	3.6	iii	iii	ii
1a.Production of NTA, dust	0.6	ii	ii	ii
3b.High pressure cleaning (diluted solutions, <2% NTA)	0.27	ii	ii	ii
1b.Production of NTA, liquid	0.12	ii	ii	ii

4.1.3.3 Consumers

Consumer exposure

Exposure of consumers to Na₃NTA results primarily from the use of different household cleaners via the dermal route.

The calculation of an aggregated dermal exposure to liquid cleaners and floor polish results in a value of 0.52 mg/kg bw/d assuming that all applications take place on one day. For spray cleaners the dermal exposure accounts for 0.44 mg/kg bw/d when glass and bath room cleaners have been applied on one day.

Exposure from pressure washers would account for 0.092 mg/kg bw/d. Residues of Na₃NTA may remain on fabrics/textiles after manufacturing and can migrate during first wearing from textile to skin. A potential dermal exposure of 1.7 µg/kg bw/d can be calculated for first wearing of clothing. This exposure will be neglected in the risk characterisation.

An inhalation exposure to aerosol of 0.6 mg/m³ was calculated for application of high-pressure cleaning. The estimated exposure levels for spray cleaner applications and by loading a dish washing machine with powder are considered to be negligible.

Oral exposure due to use of machine dishwashing products is low (0.055 µg/kg bw/d) and will therefore be neglected in the risk characterisation.

Acute toxicity

Human data on the acute toxicity of trisodium nitrilotriacetate are not available. Data on inhalation demonstrate a low acute toxicity value ($LC_{50} \geq 5 \text{ g/m}^3$) Acute dermal toxicity seems to be low as judged on the basis of a test with rabbits resulting in a minimum lethal dose of more than 10 g/kg bw for rabbits treated with a 25% aqueous substance solution. Therefore the MOS calculation of consumer scenarios is not helpful.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Irritation / Corrosivity

- ii) Human data on local irritancy are not available. Skin irritation studies with rabbits gave some evidence for mild reversible effects.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Sensitisation

A Buehler sensitisation test demonstrates a negative result. However, the test procedure was not valid due to a low concentration of the tested substance. Therefore this negative result cannot be used for risk assessment purposes.

Further testing on the sensitising properties of Na_3NTA is necessary to clarify the skin sensitising potential of the substance.

Conclusion:

- ii) There is need for further information and/or testing.

Repeated dose toxicity

Oral studies

For the derivation of a N/LOAEL all studies were considered that included the examination of toxic effects on the urinary tract (cf. 4.1.2.6 and 4.1.2.8). Studies focussing only on very specific questions like the electrolyte status were excluded. The levels of N/LOAEL were derived from NTA related toxicity (mortality increase and renal hydronephrosis at 921 g/kg

bw/d). For risk assessment purposes on non-cancer endpoints, a NOAEL of 92 mg/kg bw/d Na₃NTA.H₂O could be proposed from the 24-month cancer study (NCI, 1977).. This NOAEL is very close to the lowest dose demonstrated to be carcinogenic - at least in rats at 0.1% (100 mg/kg bw/d) Na₃NTA. Due to the fact that hyperplasias in the urinary tract were the most sensitive effects following long term exposure to NTA, the NOAEL for cytotoxic effects will not be applied for risk assessment on repeated dose toxicity. Instead the BMDL on carcinogenicity of 5.7 mg/kg bw/d taking the hyperplasias into account is used.

Inhalation

Inhalation studies on rats showed that NTA concentrations of 2000 mg/m³ (6 h/d, 4 d) were irritative to the mucosa of the respiratory tract including the nose and the eyes, whereas at 200 mg/m³ no adverse effect was reported. Prolongation of inhalation exposure to a period of 4 weeks resulted in dyspnoe in rats and guinea pigs at Na₃NTA concentrations of 340 mg/m³, the NOAEC for both species was 210 mg/m³. Systemic effects associated to the inhalation of 340 mg/m³ were diarrhea in monkeys and elevated ASAT activity and protein levels in rats indicating a minor dysfunction of liver cell metabolism. The NOAEC for systemic effects of 210 mg/m³ is derived from the rat study.

Dermal exposure

A Na₃NTA concentration applied as 2.5% Na₃NTA aqueous solution (2 ml, ≈ 50 mg/kg bw/d, 90 days) was without irritation thus representing the NOAEL for local effects on the rabbit dermis (Nixon, 1971). No indication on systemic effects was observed.

For the decision on the appropriateness of MOS, the following aspects have been considered and taken into account:

- Overall confidence in the database

The data taken into account for performing the risk characterization have been evaluated with regard to their reliability, relevance and completeness according to the TGD. The data were published in peer reviewed journals or submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognized guidelines.

The findings of all studies are not contradictory so that the judgement can be based on the database (cf. sections 4.1.2.6 and 4.1.2.8).

There are no reasons to assume limited confidence.

- Uncertainty arising from the variability in the experimental data

The studies cited in 4.1.2.6 and 4.1.2.8 allow concluding on the LOAEL of NTA related toxicity as mortality increase and renal hydronephrosis at 921 g/kg bw/d. For risk assessment purposes an oral NOAEL of 92 mg/kg bw/d Na₃NTA.H₂O could be proposed from the 24-month cancer study (NCI, 1977). This NOAEL is very close to the lowest dose demonstrated to be carcinogenic in rats at 0.1% (100 mg/kg bw/d) Na₃NTA. Due to the fact that hyperplasias in the urinary tract were the most sensitive effects following long term exposure to NTA, the NOAEL for cytotoxic effects will not be applied for risk characterisation on

repeated dose toxicity. Instead the BMDL on carcinogenicity of 5.7 mg/kg bw/d taking the hyperplasias into account is used.

There are no reasons to assume a special extent of uncertainty which have to be taken into account.

- Intra- and interspecies variation

Data on toxicokinetics of the substance give a hint on a particular variability in kinetics. Highly variable urinary excretion of NTA has been reported for several species including humans ranging from 12% of the dose (human) to 80% (female Beagle dogs) and 96% of the dose (mice). Biliary excretion is less than 1%. Whereas for animals a value of 50% absorption which represents the median of the findings in animals may be appropriate, the findings in human volunteers of 12% urinary excretion without indication that NTA is excreted via bile into the feces point to a lower absorption rate in humans. A value of 20% absorption is proposed for risk characterisation for the oral route. From the chemical structure and physicochemical data 10% absorption will be assumed as a default value for absorption via the dermal exposure.

The variability of the data on the toxicodynamics has been described in 4.1.2.6 and 4.1.2.8 and has been considered not to justify an increased MOS. For establishing the MOS, the BMDL of the most sensitive long term study on rats has been used.

- The nature and severity of the effect

The carcinogenic action of NTA in rats is proven. The effects considered as adverse effects for assessment of non-cancer endpoints are hyperplasias in the urinary tract, these effects are considered to be severe health effects.

There are no reasons to assume that the non-carcinogenic effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- The human population to which the quantitative and/or qualitative information on exposure applies

Following the exposure scenario there is no reason to assume a special risk for elderly or children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for inhalation exposure scenario

An inhalation exposure of 0.6 mg/m³ would results from high-pressure cleaning. The margin of safety between the

exposure level of 0.6 mg/m³

and the

NOAEC of 210 mg/m³

is considered to be sufficient with regard to local as well as systemic effects.

Conclusion:

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

MOS for dermal exposure scenario

Local effects

The calculation of the chronic dermal exposure of consumers due to liquid cleaners and floor polish leads to a value of 0.52 mg/kg bw/d. The margin of safety between the

exposure level of 0.52 mg/kg bw/d

and the

dermal NOAEL of 50 mg/kg bw/d

is considered to be sufficient.

Taking into account the lower values of dermal exposure due to spray cleaners (0.44 mg/kg bw/d) and due to pressure cleaners (0.092 mg/kg bw/d) these scenarios are also considered to be of no concern.

Systemic effects

The calculation of the chronic dermal exposure of consumers due liquid cleaners and floor polish results in a value of 0.52 mg/kg bw/d corresponding to an internal value of 0.052 mg/kg bw/d. The margin of safety between the

internal exposure of 0.052 mg/kg bw/d

and the

BMDL (oral) of 5.7 mg/kg bw/d

is considered to be sufficient because an internal exposure is compared with a BMDL value of a 2-year diet study.

Taking into account the lower values of dermal exposure due to application of spray cleaners (0.44 mg/kg bw/d) and of pressure cleaners (0.092 mg/kg bw/d) these scenarios are also considered to be of no concern.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Genotoxicity

Sodium salts of NTA are negative in bacterial test systems and mammalian cell cultures for gene mutations. NTA seems to have a weak potential for induction of chromosomal aberrations / micronuclei. In vivo, sodium salts of NTA did not induce genetic effects in bone marrow cells - neither micronuclei and aneuploidy nor SCE. In kidney cells, results were negative for DNA base damage and inconclusive for induction of micronuclei and DNA strand breaks. On the other hand, from in vivo germ cell tests there is limited evidence that i.p. application shows a potential for induction of aneuploidy in mouse spermatocytes at high doses. Considering the whole data basis on mammalian somatic cells in vitro and in vivo, there is no plausible evidence for in vivo mutagenicity of NTA and its sodium salts.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Carcinogenicity

NTA is carcinogenic in both sexes of rats and the mice via the oral route. Sparse data were evident for the dermal route. NTA exerts its carcinogenic activities via the urinary excretion route. From the 24-month study in rats with diet application of Na₃NTA as lowest effective tumor dose a value of 92 mg/kg bw/d can be derived (significant increase in tumors). The NOAEL in this life time study is the dose of 9 mg/kg bw/d.

Based on the actual knowledge a direct genotoxic mechanism of NTA carcinogenesis could not be demonstrated. It is thought that NTA might be operative at target sites by threshold-based actions. As starting point for quantitative risk characterisation on carcinogenicity it is proposed to use a benchmark dose lower bound (BMDL) of 5.7 mg/kg bw/d where a treatment-related increase in response of 10% could not be expected within the 95% confidence interval.

NTA is classified as category 3 carcinogen and labelled with Xn, R 40.

MOS for dermal exposure scenario

The calculation of the chronic dermal exposure of consumers due liquid cleaners and floor polish leads to 0.52 mg/kg bw/d corresponding to an internal value of 0.052 mg/kg bw/d. The margin of safety between the

internal exposure level of 0.052 mg/kg bw/d

and the

BMDL (oral) of

5.7 mg/kg bw/d

is considered to be sufficient because an internal exposure is compared with a BMDL value of a 2-year diet study.

Taking into account the lower values of dermal exposure due to spray cleaners (0.44 mg/kg bw/d) and due to pressure cleaners (0.092 mg/kg bw/d) these scenarios are also considered to be of no concern.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Reproductive toxicity

From the available information it appears that NTA at dietary levels of approximately 450 mg/kg bw/d did not adversely affect reproductive performance and capability through two successive generations in rats. Any specific teratogenic potential and/or impairment of embryo/fetal development was not indicated from the data of that study for rats at dietary levels of approximately 450 mg/kg bw/d and for rabbits at gavage doses of 250 mg/kg bw/d. Higher dose levels up to and including clearly parentally toxic doses have not been investigated so far. Taken together, fertility and developmental toxicity are not considered to be relevant endpoints.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

4.1.3.4 Man exposed indirectly via the environment

Indirect exposure via the environment is calculated using data for oral intake via food and drinking water. Following the local scenario data (at a point source) a total daily dose in the order of 0.004 mg/kg bw/d is calculated with the main contributions of the DOSEdrinking water and DOSEfish with fractions of 37% and 63%, respectively. Following the data for the regional scenario, the total daily dose amounts to 3.3×10^{-5} mg/kg bw/d.

Repeated dose toxicity

As starting point for quantitative risk characterisation on repeated dose toxicity the benchmark dose lower bound (BMDL) on carcinogenicity of 5.7 mg/kg bw/d taking into account the hyperplasias is used (cf. 4.1.3.3)

MOS for the oral exposure scenario: Man exposed indirectly via the environment

Local scenario

The highest total dose calculated for a point source was 0.004 mg/kg bw/d (local scenario). The margin of safety between the

exposure level of 0.004 mg/kg bw/d

and the

BMDL (oral) of 5.7 mg/kg bw/d

is judged to be sufficient.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Regional scenario

The total dose was calculated to be 3.3×10^{-5} mg/kg bw/d (regional scenario). The margin of safety between the

exposure level of 3.3×10^{-5} mg/kg bw/d

and the

BMDL (oral) of 5.7 mg/kg bw/d

is judged to be sufficient.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Genotoxicity

Based on the available data mutagenicity is not of concern (cf. 4.1.3.3)..

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Carcinogenicity

NTA is carcinogenic in rats and the mice via the oral route. From the 24-month study in rats with diet application of Na₃NTA the NOAEL of 9 mg/kg bw/d was derived. Based on the

actual knowledge a direct genotoxic mechanism of NTA carcinogenesis could not be demonstrated. It is thought that NTA might be operative at target sites by threshold-based actions. As starting point for quantitative risk characterisation on carcinogenicity the benchmark dose lower bound (BMDL) of 5.7 mg/kg bw/d is used (cf. 4.1.3.3).

Local scenario

The highest total dose calculated for a point source was 0.004 mg/kg bw/d (local scenario). The margin of safety between the

exposure level of 0.004 mg/kg bw/d

and the

BMDL (oral) of 5.7 mg/kg bw/d

is judged to be sufficient.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Regional scenario

The total dose was calculated to be 3.3×10^{-5} mg/kg bw/d (regional scenario). The margin of safety between the

exposure level of 3.3×10^{-5} mg/kg bw/d

and the

BMDL (oral) of 5.7 mg/kg bw/d

is judged to be sufficient.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Reproductive toxicity

From the available data it appears that NTA at dietary levels of approximately 450 mg/kg bw/d did not adversely affect reproductive performance and capability through two successive generations in rats. Any specific teratogenic potential and/or impairment of embryo/fetal development was not indicated from the data of that study for rats at dietary levels of approximately 450 mg/kg bw/d and for rabbits at gavage doses of 250 mg/kg bw/d. Taking into account the low exposure values for humans via the environment the substance is considered to be without concern in relation to reproduction toxicity.

Conclusion:

- ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

4.1.3.5 (Combined exposure)

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

See chapter 4.1.1.1

4.2.1.2 Consumer exposure

4.2.1.3 Indirect exposure via the environment

4.2.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

Trisodium nitrilotriacetate is not explosive.

4.2.2.2 Flammability

Trisodium nitrilotriacetate is not flammable.

4.2.2.3 Oxidising potential

Due to its chemical structure, trisodium nitrilotriacetate is not expected to possess any oxidizing properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

not applicable

4.2.3.2 Consumers

Man exposed indirectly via the environment.

4.2.3.3 Man exposed indirectly via the environment

5 CONCLUSIONS / RESULTS

- () i) There is need for further information and/or testing
- (X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already
- (X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusion:

Environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

In the present risk assessment production and use of Na₃NTA are examined. For all life-cycle steps, the PEC/PNEC ratios are below 1. Therefore, a risk for the environment is not expected.

Human Health

Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Concern is derived for repeated dose toxicity and carcinogenicity. Especially dermal exposure (with a critical exposure level of 2.85 mg/kg/day) has to be reduced for scenario 2 (use of Na₃NTA in formulation process) and 3 (high pressure cleaning), and inhalation exposure (with a critical exposure level of 2 mg/m³) has to be reduced for scenario 1 (production of NTA) and 2b (use of Na₃NTA in formulation process without LEV).

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

For the other toxicological endpoints the risk orientated conclusions result in no concern with the consequence that risk reduction measures are of low priority.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Man exposed indirectly via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

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APPENDIX 1

The evaluation of the skin sensitisation potential of NTA predicted by TOPKAT and DerekfW and a Weight of Evidence approach including all data.

The Netherlands 12 September (2007)

Introduction

For NTA a Buehler test is available, which was negative. The sensitivity of this test can be limited because the dermal barrier is intact. In the Buehler test with NTA no skin irritation was found in the induction phase. Therefore it is not clear whether dermal penetration had occurred and if the immune system was reached. The German CA proposed to carry out an LLNA test. NL reasoned that further testing was not required because the reactivity of the chemical was limited. This reasoning was based on expert judgement, DerekfW and TOPKAT models.

This latter information is now more documented and more explanation is given to the Weight of Evidence (WoE) approach to draw a conclusion for the skin sensitisation potential.

Methods

When gathering all data available the methods and models and their outcome will need to be documented and structured in a way that the outcomes can be compared. Two tables have been made to structure this information.

Results

In table 1, the methods and models are documented and in table 2 the outcome of the methods and models are documented.

Weight of Evidence approach for skin sensitisation (NTA)

The outcome of the Buehler test was negative, which is almost of sufficient weight for the absence of the skin sensitisation potential. The bioavailability of NTA for sensitisation is limited because it is a salt and ionised chemicals have limited dermal penetrations (expert judgment). At the other hand the MW of 257.1 g/mol does not exclude dermal penetration. According the DerekfW the chemical reactivity and so the binding to proteins is limited. Generally speaking the outcome of DerekfW for the absence of sensitisation has limited

weight, because negative findings usually have to be considered out of the applicability domain. However, viewing the alerts present in DerekfW this type of chemicals may be considered negative. Protein binding is considered limited, because the ester-salt is not activated and the amine is tertiary which may be sterically hindered (expert judgment). The chemical is within the TOPKAT domain and provides an analogue which is negative. Though the TOPKAT models may have some uncertainty because of lack of mechanistic reasoning, the analogue approach can add to the overall impression that the chemical is not a sensitizer.

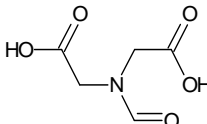
Conclusion

It can be concluded that despite the less sensitive Buehler, the limited reactivity and an analogue from TOPKAT strengthen the absence of skin sensitisation potential. Therefore we do not think an LLNA test is needed.

Table 1 Information on Buehler test, DerekfW and TOPKAT

Methods/ Models used	Endpoint	Key parameter for evaluation	Domain of Applicability	Validation	Mechanisms
Buehler test	Sensitisation Number of animals sensitised	Sensitisation = Bioavailability x Reactivity x Immunological activity	All dermal bioavailable compounds	OECD guideline Sensitivity more limited for weak sensitizers	Dermal penetration is crucial for showing reactivity. Reactive chemicals that can provoke an immunological activity.
DerekfW vs 8.0	GPMT, LLNA, Buehler and human experience	Structural alerts that indicate binding to proteins and can cause hapten formation	It identifies at least 61 structural alerts for sensitisation	Positives are in the domain Negatives may or may not be in the domain	Those that are identified in the tests mentioned for the endpoint.
TOPKAT	GPMT, LLNA, Buehler and human experience	Chemical categories and chemical connectivity descriptors. Probability < 0.3 absence > 0.7 presence and 0.3-0.7 equivocal sensitisation potential	Indicated by the program	Internal validation only. Sensitivity and specificity > 80% No of compounds between 200- 300	Those that are identified in the test
Expert judgement	Chemical reactivity	Qualitative evaluation on electro- philicity	Electrophile chemicals	No validation or uncertainty for specific classes of chemicals	Electrophilicity is one of the sensitisation inducing parameters
Expert judgment	Dermal penetration	Qualitative	Very low or very well dermal available compounds	No validation or uncertainty indication	Salts being ionised chemical have limited dermal penetration

Table 2 Outcome of methods and models for NTA

Outcome of methods and models NTA	Endpoint	Outcome of measured parameter	Outcome of applicability domain	Uncertainty of the outcome	Underlying mechanisms
Buehler test	0/20 chemicals sensitised	Negative for sensitisation	Borderline applicability domain	OECD guideline	Absence of skin irritation due to absence of bioavailability or reactivity
Derekw vs 8.0	Potential sensitiser	No alert fired	Only alpha beta unsaturated esters and diamines are considered positive. Therefore the chemical is on the borderline of the applicability domain	Some validation studies are present	Only activated esters and amines are considered sufficiently reactive
TOPKAT*	Sensitisation potential is considered zero	Aliphatics Probability 0, which means the absence of sensitisation	Within the domain Closest structural analogue is: 	Closest analogue is negative SAR and QSAR in Env. Res, 1994: 159	Unknown
Expert judgment	Reactivity is limited	Electrophilicity Radicals formation not expected.	Not identified	Not identified	
Expert judgment	Bioavailability is limited but not excluded	Salt limits and MW indicates dermal penetration	Not identified	Not identified	

* The acid has been predicted not the salt.