NITRILOTRIACETIC ACID AND ITS SALTS

1. Chemical and Physical Data

1.1 Synonyms

Nitrilotriacetic acid (NTA)

Chem. Abstr. Services Reg. No.: 139-13-9
(Replaced CAS Reg. Nos 26627-44-1, 26627-45-2 and 80751-51-5)
Chem. Abstr. Name: Glycine, *N,N*-bis(carboxymethyl)-
IUPAC Systematic Name: Nitrilotriacetic acid
Synonyms: Nitrilo-2,2′,2″-triacetic acid; triglycine; triglycollamic acid; α,α′, α″-trimethylaminetricarboxylic acid

Nitrilotriacetic acid, sodium salt (unspecified)

Chem. Abstr. Services Reg. No.: 10042-84-9
Chem. Abstr. Name: Glycine, *N,N*-bis(carboxymethyl)-, sodium salt
IUPAC Systematic Name: Nitrilotriacetic acid, sodium salt
Synonyms: Sodium aminotriacetate; sodium nitriloacetate; sodium nitrilotriacetate; sodium NTA

Nitrilotriacetic acid, monosodium salt (NTA, monosodium salt)

Chem. Abstr. Services Reg. No: 18994-66-6
Chem. Abstr. Name: Glycine, *N,N*-bis(carboxymethyl)-, monosodium salt
IUPAC Systematic Name: Sodium dihydrogen nitrilotriacetate
Synonyms: Monosodium nitriloacetate; monosodium nitrilotriacetate; NaNTA

Nitrilotriacetic acid, disodium salt (NTA, disodium salt)

Chem. Abstr. Services Reg. No.: 15467-20-6
Chem. Abstr. Name: Glycine, *N,N*-bis(carboxymethyl)-, disodium salt
IUPAC Systematic Name: Disodium hydrogen nitrilotriacetate
Synonyms: Disodium nitrilotriacetate; Na_2NTA

Nitrilotriacetic acid, disodium salt, monohydrate (NTA disodium salt, monohydrate)

Chem. Abstr. Services Reg. No.: 23255-03-0
Chem. Abstr. Name: Glycine, *N,N*-bis(carboxymethyl)-, disodium salt, monohydrate
IUPAC Systematic Name: Disodium hydrogen nitrilotriacetate, monohydrate
Synonyms: Acetic acid, nitrilotri-, disodium salt, monohydrate; disodium nitrilotriacetic acid monohydrate; $Na_2NTA.H_2O$

Nitrilotriacetic acid, trisodium salt (NTA, trisodium salt)

Chem. Abstr. Services Reg. No.: 5064-31-3
(Replaced CAS Reg. No. 37291-81-9)
Chem. Abstr. Name: Glycine, *N,N*-bis(carboxymethyl)-, trisodium salt
IUPAC Systematic Name: Trisodium nitrilotriacetate
Synonym: Na_3NTA

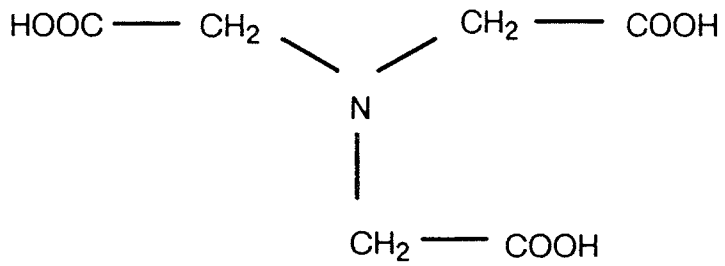
Nitrilotriacetic acid, trisodium salt, monohydrate (NTA, trisodium salt, monohydrate)

Chem. Abstr. Services Reg. No.: 18662-53-8
Chem. Abstr. Name: Glycine, *N,N*-bis(carboxymethyl)-, trisodium salt, monohydrate
IUPAC Systematic Name: Trisodium nitrilotriacetate, monohydrate
Synonyms: $Na_3NTA.H_2O$; trisodium salt of nitrilotriacetic acid, monohydrate

Many other salts of NTA have been reported, and selected examples are listed in Table 1. Chemical and physical properties have not been reported for most of these.

1.2 Structural and molecular formulae and molecular weights

NTA



$C_6H_9NO_6$ Mol. wt: 191.14

NTA, monosodium salt

$C_6H_8NO_6 \cdot Na$ Mol. wt: 213.14

NTA, disodium salt

$C_6H_7NO_6 \cdot 2Na$ Mol. wt: 235.13

NTA, disodium salt, monohydrate

$C_6H_7NO_6 \cdot 2Na \cdot H_2O$ Mol. wt: 253.11

NTA, trisodium salt

$C_6H_6NO_6 \cdot 3Na$ Mol. wt: 257.13

NTA, trisodium salt, monohydrate

$C_6H_6NO_6 \cdot 3Na \cdot H_2O$ Mol. wt: 275.11

Table 1. Examples of nitrilotriacetic acid salts and complexes

CAS No.	Common name
1188-47-2	NTA, copper(2+) salt (1:1)
1188-48-3	NTA, magnesium salt (1:1)
2399-81-7	NTA, beryllium salt (1:1)
2399-83-9	NTA, barium salt (1:1)
2399-85-1	NTA, tripotassium salt
2399-86-2	NTA, dipotassium salt
2399-88-4	NTA, potassium magnesium salt (1:1:1)
2399-89-5	NTA, potassium strontium salt (1:1:1)
2399-94-2	NTA, calcium salt (1:1)
2455-08-5	NTA, calcium potassium salt (1:1:1)
3130-95-8	NTA, scandium (3+) salt (1:1)
5798-43-6	NTA, disodium salt, compound with oxo(dihydrogen nitriloacetato)bismuth sodium salt (3:1)
10413-71-5	NTA, erbium(3+) salt (3:1)
14695-88-6	NTA, compound with iron chloride (FeCl3)
14981-08-9	NTA, calcium salt
15414-25-2	NTA, yttrium (3+) salt (1:1)
15844-52-7	NTA, copper(2+) complex
15934-02-8	NTA, monoammonium salt
16448-54-7	NTA, iron(3+) complex [replaced: 5905-54-4; 107288-49-3]
18105-03-8	NTA, mercury(2+) salt (2:3)
18432-54-7	NTA, cadmium(2+) complex
18946-94-6	NTA, neodymium(3+) salt (1:1) [alternative: 3438-06-0]
18983-72-7	NTA, beryllium potassium salt (1:1) [alternative: 2399-87-3]
19010-73-2	NTA, aluminium(3+) complex
19456-58-7	NTA, indium(3+) complex
22965-60-2	NTA, nickel(3+) complex
23319-51-9	NTA, cobalt(3+) complex
23555-96-6	NTA, potassium strontium salt (2:4:1)
23555-98-8	NTA, calcium potassium salt (2:1:4)
25817-24-7	NTA, potassium salt
28444-53-3	NTA, monopotassium salt
28927-38-0	NTA, holmium salt
29027-90-5	NTA, cerium salt
29507-58-2	NTA, zinc(3+) complex sodium salt [replaced: 26856-43-9]
32685-17-9	NTA, triammonium salt
34831-02-2	NTA, copper(2+) hydrogen complex
34831-03-3	NTA, nickel(2+) hydrogen complex
36711-58-7	NTA, manganese salt
46242-44-8	NTA, antimony(3+) complex
50648-02-7	NTA, tricadmium(2+) complex

Table 1 (contd)

CAS No.	Common name
53108-47-7	NTA, copper(2+) complex sodium salt
53108-50-2	NTA, cobalt(3+) hydrogen complex
53818-84-1	NTA, tin(2+) salt
60034-45-9	NTA, calcium sodium salt (1:1:1)
61017-62-7	NTA, iron(2+) complex sodium salt (1:1:1)
62979-89-6	NTA, calcium salt (2:3)
71264-32-9	NTA, diammonium salt
71484-80-5	NTA, copper(2+) complex ammonium salt
72629-49-3	NTA, dilithium salt
73772-91-5	NTA, magnesium salt
79849-02-8	NTA, lead(2+) salt (1:1)
79915-08-5	NTA, lead(2+) potassium salt (1:1:1)
79915-09-6	NTA, lead(2+) salt (2:3)
86892-89-9	NTA, disodium ammonium salt
92474-39-0	NTA, trisilver salt
92988-11-9	NTA, strontium sodium salt

1.3 Chemical and physical properties of the pure substance

NTA

(*a*) *Description*: White crystalline powder (Anderson *et al.*, 1985; W.R. Grace & Co., 1985)

(*b*) *Melting-point*: Decomposes at 246°C (Sadtler Research Laboratories, 1980; Aldrich Chemical Co., 1988)

(*c*) *Spectroscopy data*: Infrared (prism [5940, 13213]; grating [18901]) and nuclear magnetic resonance spectral data have been reported (Sadtler Research Laboratories, 1980; Pouchert, 1981, 1983, 1985).

(*d*) *Solubility*: 1.5 g/l water at 25°C (Anderson *et al.*, 1985; W.R. Grace & Co., 1985)

(*e*) *pH of saturated aqueous solution*: 2-3 (W.R. Grace & Co., 1985)

(*f*) *Reactivity*: Forms water-soluble complexes with many metal ions (chelates); reacts with strong oxidizing agents such as hypochlorite, chlorine and ozone (Anderson *et al.*, 1985)

NTA, disodium salt

(*a*) *Melting-point*: >300°C (Aldrich Chemical Co., 1988)

(*b*) *Spectroscopy data*: Infrared (prism-FT [566B]) spectral data have been reported (Pouchert, 1985).

(*c*) *Reactivity*: Forms water-soluble complexes with many metal ions (chelates); reacts with strong oxidizing agents such as hypochlorite, chlorine and ozone (Anderson *et al.*, 1985)

NTA, trisodium salt, monohydrate

(*a*) *Description*: White crystalline powder (Anderson *et al.*, 1985; Monsanto Co., 1985)

(*b*) *Melting-point*: Decomposes at 340°C (Monsanto Co., 1985)

(*c*) *Spectroscopy data*: Infrared and nuclear magnetic resonance spectral data have been reported (Pouchert, 1981, 1983, 1985).

(*d*) *Density*: 1.782 g/cm^3 (Monsanto Co., 1985)

(*e*) *Solubility*: 50 g/100 g water at 25°C (Anderson *et al.*, 1985; Monsanto Co., 1985)

(*f*) *pH of 1% solution at 25°C*: 10.6-11.0 (Monsanto Co., 1985)

(*g*) *Reactivity*: Forms water-soluble complexes with many metal ions (chelates); reacts with strong oxidizing agents such as hypochlorite, chlorine and ozone (Anderson *et al.*, 1985)

Salt and complex formation

As a carboxylic acid, NTA forms simple salts with some metal ions (e.g., sodium, potassium), but the association between NTA and other metals ions that have available additional coordination sites for binding may involve more than one of the NTA functional groups. NTA in its fully ionized form [$N(CH_2CO_2^-)_3$; NTA^{3-}] has four functional groups—the three carboxylates and one amine group—that are available for complexing with a metal ion. This phenomenon, in which more than one of the NTA functional groups is involved in binding with a metal ion, is known as chelation. For example, the structure of the Fe^{3+}/NTA^{3-} complex involves simultaneous binding of the three carboxylate groups and the amine group to the Fe^{3+} ion. Other metal ions show intermediate degrees of complexation with NTA in its ionized (salt) form in solution.

Complexes are formed between metal ions and NTA when metal salts and NTA salts are combined in aqueous solutions. The proportion of metal ions complexed in a given solution depends on a number of factors, and the complex is always in equilibrium with uncomplexed metal ion and NTA. In aqueous solution, the extent of protonation of the NTA carboxylate groups varies with pH ($pKA_1 = 1.89$, $pKA_2 = 2.49$, $pKA_3 = 9.73$); protonation competes with metal ion complexation. The extent of metal complex formation in solution depends on the concentrations of the ionized forms of NTA and the metal ion, and on the formation constant (inverse of dissociation constant) of the complex. Table 2 gives the formation constants for several common metal ions. As can be seen, the weakest NTA/metal ion complexes are formed with the alkaline earth ions, Mg^{2+} and Ca^{2+}, and the strongest complexes are formed with Hg^{2+} and Fe^{3+} (Martell & Smith, 1974; Anderson *et al.*, 1985).

Table 2. Metal ion/NTA^{3-} formation constants at 25 °C[a]

Metal	log K
Mg^{2+}	5.47
Ca^{2+}	6.39
Mn^{2+}	7.46
Fe^{2+}	8.33[b]
Cd^{2+}	9.78
Co^{2+}	10.38
Zn^{2+}	10.66
Pb^{2+}	11.34
Ni^{2+}	11.50
Cu^{2+}	12.94
Hg^{2+}	14.6
Fe^{3+}	15.9

[a]From Martell & Smith (1974)
[b]At 20 °C

1.4 Technical products and impurities

Trade Names:

NTA: Chel 300; Complexon I; Hampshire® NTA acid; IDRANAL® I; Titriplex I; Versene NTA acid

NTA, sodium salt: Chelest NTA

NTA, disodium salt: Chelest NTB

NTA, trisodium salt: Chemcolox 365 Powder; Hampshire® NTA 150; Masquol NP 140; Syntron A; Trilon A; Trilon A 50; Versene NTA 150; Versene NTA 335

NTA, trisodium salt, monohydrate: Hampshire® NTA Na3 Crystals; NTA Powder; Trilon® A92

NTA and its sodium salts are commercially available in several products with the following specifications: (i) crystals (white granular powder) of NTA, trisodium salt, monohydrate; purity, 98.5% min (W.R. Grace & Co., 1985) and 99% (Aldrich Chemical Co., 1988); (ii) aqueous solution (clear, pale straw-coloured liquid) of NTA, trisodium salt, 40% min); specific gravity, 1.30-1.33 at 25 °C; and (iii) crystalline form (free-flowing, white, crystalline powder) of NTA; purity, 99% (W.R. Grace & Co., 1985). Another manufacturer markets NTA, trisodium salt, monohydrate with a reported purity of 100%; density, 1.782 g/cm^3 (Monsanto Co., 1985). Reagent-grade NTA, disodium salt is commercially available with a purity of >99% (Aldrich Chemical Co., 1988). Reagent-grade NTA is also available with the

following specifications: purity, 99.5% min; calcium, 0.002% max; iron, 0.0005% max; potassium, 0.001% max; magnesium, 0.001% max; sodium, 0.02% max; chloride, 0.02% max; and sulfate, 0.01% max (Riedel-de Haen, 1984).

Analysis of commercial NTA, trisodium salt, monohydrate has shown the following impurities: sodium cyanide, 4 ppm (mg/kg); sodium hydroxide, 0.3%; sodium carbonate, 0.4%; primary and secondary amines, approximately 0.2%; iminodiacetic acid, 0.2%; potassium, 6 ppm; zinc, 2 ppm; copper, 1 ppm; iron, < 10 ppm; and lead (see IARC, 1987a), 1-2 ppm (Anderson *et al.*, 1985). The typical composition of another commercial product (also NTA trisodium salt, monohydrate) was given as: NTA, trisodium salt, monohydrate, 99%; iminodiacetic acid, disodium salt, < 0.2%; sodium hydroxide, < 0.5%; sodium carbonate, < 0.5%; ammonia, < 70 ppm; formaldehyde (see IARC, 1987b), < 5 ppm; and sodium cyanide, < 5 ppm (Gesellschaft Deutscher Chemiker, 1987).

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(*a*) *Production*

NTA was first synthesized by Heintz in 1862, and its properties and chemistry were described in 1865 (Anderson *et al.*, 1985).

First commercial production of NTA occurred in Europe in the 1930s. Subsequently, it was found that ethylenediaminetetraacetate (EDTA) could be synthesized at a lower cost, and, in a relatively short period of time, EDTA replaced NTA in most commercial applications. With the later development of an efficient, low-cost process for manufacturing highly pure NTA products, NTA has again become competitive for many applications (W.R. Grace & Co., 1985).

NTA, trisodium salt, monohydrate is synthesized commercially by the reaction of formaldehyde, hydrocyanic acid and sodium hydroxide in water (Anderson *et al.*, 1985). The trisodium salt, isolated from aqueous solution as the monohydrate, can be converted to other forms by dehydration or acidification.

Combined European production capacity for NTA in the early 1980s was estimated at 50 000 tonnes, and overall annual consumption of NTA and its salts estimated at about 20 000 tonnes. About 8000 tonnes of NTA and its salts were produced in the Federal Republic of Germany in 1984 (Gesellschaft Deutscher Chemiker, 1987). They have also been manufactured in France, the Netherlands, Spain, Sweden and the UK. US production in the early 1980s was about 30 000 tonnes per year, most of which was exported. In that period, Japan produced less than 1000 tonnes per year and imported 1000-2000 tonnes. Canada imported 7400 tonnes in 1984 but only 420 tonnes in 1987 due to increased use of zeolite in detergent formulations; NTA is also produced in Canada, but production figures were not available (Universities Associated for Research and Education in Pathology, 1985).

(b) Use

NTA has numerous commercial applications as a metal ion chelator, including principally its use in cleaning products, industrial water treatment, textile preparation and metal finishing. It has also been used to a lesser extent in the pulp and paper industry, in rubber processing, in photographic products, in the electrochemical industry, in the tanning of leather, and in cosmetics (Anderson *et al.*, 1985; Universities Associated for Research and Education in Pathology, 1985).

The major use of NTA, as the trisodium salt, has been in detergent systems as a chelating agent and as a laundry detergent builder. NTA was originally proposed as a substitute for phosphates in detergents, when the eutrophic effect of phosphates on the aquatic environment was recognized. It has been accepted for use as an ingredient in domestic detergent products in at least 16 countries and has actually been used in detergents in Canada and several European countries since the early 1970s. NTA is also used to reduce fabric yellowing by hypochlorite bleach and to increase the effectiveness in hard water of liquid detergent-sanitizer formulations based on quaternary ammonium germicides (Universities Associated for Research and Education in Pathology, 1985; W.R. Grace & Co., 1985; Wendt *et al.*, 1988).

NTA can chelate metal ions that commonly cause water 'hardness' (Ca^{2+} and Mg^{2+}) and is widely used to control precipitation and scaling of salts of these ions, for example, in boiler feedwater. Various salts of NTA have been used to remove scale. Since the sodium-calcium NTA complex is relatively insoluble, it can form a coating on the scale and retard further scale formation. Iron oxide deposits such as mill scale are removed with NTA, ammonium salts in the alkaline pH range, resulting in a degreased and cleaned surface (Anderson *et al.*, 1985; W.R. Grace & Co., 1985).

Trace metals in dye processing are often responsible for uneven dyeing of stock, piece and continuous goods by forming interfering metal lakes, which result in streaking and dulling of shades. Hardness can be controlled and heavy metals eliminated by incorporating NTA in the processing. NTA is also used in scouring and fulling operations, in peroxide bleaching and in desizing operations (Anderson *et al.*, 1985; W.R. Grace & Co., 1985).

The addition of NTA to conventional alkaline metal cleaners assists in dissolving water-insoluble metal oxides and hydroxides which are formed when metals corrode (Anderson *et al.*, 1985; W.R. Grace & Co., 1985).

(c) Regulatory status and guidelines

The US Food and Drug Administration (1987) has approved the use of NTA, trisodium salt as an additive to boiler water used in the preparation of steam that will come into contact with food. It may not exceed 5 ppm (mg/l) in boiler feedwater and may not be used when steam will be in contact with milk and milk products.

One US manufacturer has adopted for their operations a workplace exposure limit to NTA powder (as total dust) of 1 mg/m^3 as an 8-h time-weighted average and 2 mg/m^3 as a short-term exposure level (Monsanto Co., 1985).

2.2 Occurrence

(*a*) *Natural occurrence*

NTA, its salts and its complexes are not known to occur as natural products.

(*b*) *Occupational exposure*

About 2600 workers in the USA may be potentially exposed to NTA salts during their production or during the formulation of detergents. In the production of NTA, people loading hopper cars have the highest potential exposure. In one study, mean airborne levels of NTA in the workplace during the production of NTA were 0.033 mg/m^3 in the normal line area (US Environmental Protection Agency, 1979; Universities Associated for Research and Education in Pathology, 1985), but 0.82 mg/m^3 in the hopper-car loader area (US Environmental Protection Agency, 1979).

Increases recorded in the concentrations of NTA in influent sewage in several countries imply that the potential exposure of sewage treatment workers to this compound has risen steadily since the early 1970s (Anderson *et al.*, 1985).

(*c*) *Water and sediments*

(i) *Sewage treatment systems*

In an environmental monitoring programme carried out in 1971-75, concentrations of NTA and certain metals were measured in Canadian wastewaters and streams. During 1971 and 1972, the average level of NTA in detergents in Canada was 6%; between 1973 and 1975, it had increased to 15%. The levels of NTA that were found in sewage influents and effluents during the two periods are shown in Table 3. In 13 cities, the levels of NTA that were found in receiving streams above the sewage outfall ranged from 0 to 190 µg/l during 1971-72 and 0 to 283 µg/l during 1973-75; the levels below the sewage outfall ranged from 0 to 340 and from 0 to 3364 µg/l in the two periods, respectively (Woodiwiss *et al.*, 1979).

Table 3. Mean concentrations (mg/l) of NTA in 13 Canadian sewage influents and effluents[a]

Treatment	1971-72		1973-75	
	Influent	Effluent	Influent	Effluent
Activated sludge	2.14	0.40	3.80	0.60
Trickling filter	1.76	1.27	5.16	3.22
Primary	1.14	0.75	3.19	2.98
No treatment	1.09	NA	1.75	NA
Total geometric mean	1.73		3.62	

[a]From Woodiwiss *et al.* (1979)

NA, not applicable

Concentrations of NTA in 1980 at four sewage treatment plants in the Netherlands ranged from 37 to 113 µg/l in influent samples and from 6 to 21 µg/l in effluent samples (Games *et al.*, 1981). In a later environmental monitoring programme conducted at the same four treatment plants, NTA concentrations ranged from 80 to 254 µg/l in influent samples and from 4 to 48 µg/l in effluent samples (Anderson *et al.*, 1985).

In an environmental monitoring programme conducted in Indiana, USA, in 1979-83, the concentrations of NTA were measured before and after NTA was incorporated into laundry detergents at five types of site: in wastewaters before and after treatment, in river water above and below wastewater outfalls, and in finished drinking-water drawn from rivers receiving large amounts of NTA (Table 4). Concentrations of metals were also measured, in order to determine whether NTA used in laundry detergents affected metal concentrations in wastewater, river water or drinking-water; no effect was observed (Wendt *et al.*, 1988).

Table 4. Concentrations (mg/l) of NTA in wastewaters, river waters and drinking-water in Indiana, USA[a]

Water type	No. of samples	Before NTA use		During NTA use	
		Mean	Range	Mean	Range
Wastewaters					
Influent	4	0.025	0.010-0.069	0.789	0.385-2.314
Effluent	4	0.013	0.003-0.036	0.137	0.021-0.639
River waters	9	0.002	< 0.001-0.006	0.008	0.003-0.031
Below wastewater outfall		-		0.012	
Drinking-water	4	0.001	< 0.001-0.003	0.004	0.002-0.008

[a]From Wendt *et al.* (1988)

-, not measured

(ii) *Drinking-water*

Concentrations of NTA in domestic drinking-water supplies have been monitored in several studies; the results are summarized in Table 5.

2.3 Analysis

Methods for the analysis of NTA in the environment have been reviewed (Kirk & Lester, 1981; Kirk *et al.*, 1982). Selected methods are presented in Table 6.

Table 5. Concentrations of NTA in domestic water supplies[a]

Area	Date	Drinking-water		No. of samples	Concentration (μg/l)	Reference
		Type	Source			
USA						
New York (Upstate)	1981–83	Municipal	Surface or groundwater	46 24	ND < 5	Procter & Gamble Co. (1983a)
Indiana	1981–83	Municipal	Surface water	152	Average, 4	Procter & Gamble Co. (1981, 1982, 1983b)
Canada						
Nationwide	1972–75	Municipal	Surface water	650 51	ND 10–80	Matheson (1977)
	1972–75	Municipal	Groundwater	77 1	ND Detected, no level reported	Matheson (1977)
	1976–77	Municipal	Surface water		National average, 2.8	Malaiyandi *et al.* (1979)
Ottawa–Carleton, Ontario	1976–77	Private	Groundwater	20 1	ND 16.9	Malaiyandi *et al.* (1979)
	1983	Private	Groundwater	18 1	ND 2.7	Procter & Gamble Co. (1983c)

Table 5 (contd)

Area	Date	Drinking-water		No. of samples	Concentration (μg/l)	Reference
		Type	Source			
Finch, Ontario	1972	Private	Groundwater	47 21	< 10 15–250	Matheson (1977)
	1972–73	Private	Groundwater	68 4	< 10 70	Matheson (1977)
	1973	Private	Groundwater	5 1	< 10 3900	Matheson (1977)
	1983	Private	Groundwater	13 7 2	ND < 5 5.2 and 14	Procter & Gamble Co. (1983c)
Port Kells, British Columbia	1983	Private	Groundwater	18 2	ND 2.5 and 2.6	Procter & Gamble Co. (1983c)

[a]Reviewed by Anderson *et al.* (1985)

ND, none detected

Table 6. Methods for the analysis of NTA in water

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Water and sewage effluents	Add ion-exchange resin; stir; filter; add zinc-Zincon reagent	Colorimetric	0.2 mg/l (as triNa salt)	Thompson & Duthie (1968)
Sewage effluents	Adjust pH to 2.0-2.5 with hydrochloric acid; add ferric nitrate solution; heat; add ammonium hydroxide/ammonium nitrate to raise pH to 3.9-4.1; cool; filter; add phenanthroline reagent	Colorimetric	5 mg/l	Swisher *et al.* (1967)
Water	Deaerate with nitrogen gas; add indium (III) nitrate	DPP	0.3 ppm	Haberman (1971)
Stream, sea- and sewage water	Add hydroxylamine sulfate solution; heat; cool; add acetate (chloride for saline solutions) electrolyte and bismuth nitrate solution; deaerate	DPP	0.01 mg/l	Hoover (1973)
Water	Acidify with ascorbic and nitric acid; deaerate with nitrogen gas; add bismuth nitrate; determine before and after addition of bismuth	DPP	0.05 mg/l	Haring & van Delft (1977)
Water	Convert to corresponding tri-*n*-butyl ester	GC/FID	Not reported	Warren & Malec (1972)
Tap water and sewage effluents	Isolate by anion-exchange chromatography; convert to tri-*n*-butyl ester	GC/FID	1 ppb (µg/l)	Aue *et al.* (1972)
Tap water and sewage effluents	Convert to tri-*n*-butyl ester	GC/NPD, GC/MS/SIM	1 µg/l	Games *et al.* (1981)

[a]DPP, differential pulse polarography; GC/FID, gas chromatography/flame ionization detection; GC/NPD, gas chromatography/nitrogen phosphorus detection; GC/MS/SIM, gas chromatography/mass spectrometry/selective ion monitoring

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

NTA and its salts

(*a*) *Dietary administration*

NTA

Mouse: Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were fed 7500 or 15 000 (maximum tolerated dose) ppm (mg/kg) commercial-grade NTA (purity, 99.5%) in the diet for 18 months and were killed at 21 months. Groups of 20 male and 20 female mice served as controls. More weight loss was observed in high- and low-dose females and in high-dose males than in controls; survival was comparable in treated and control animals of each sex. Hydronephrosis was detected in 8/44 high-dose males and 12/50 high-dose females, and animals of each sex had increased incidences of renal tumours, mostly adenocarcinomas: males—control, 0/20; low-dose, 5/49; high-dose, 24/44 ($p < 0.001$; χ^2 for 2×3 contingency table); females—control, 0/20; low-dose, 0/39; high-dose, 4/50 ($p = 0.041$, test for linear trend) (National Cancer Institute, 1977).

Rat: Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were fed 7500 or 15 000 (maximum tolerated dose) ppm (mg/kg) commercial-grade NTA (99.5% pure) in the diet for 18 months and were killed at 24 months. Groups of 20 males and 20 females served as controls. A modest, dose-related decrease in body weight gain was observed; survival was comparable in treated and control animals of each sex. Renal interstitial fibrosis and tubular dilatation were found frequently. Increases were observed in the incidences of urinary-tract tumours, mainly tubular-cell adenomas and carcinomas, in males: control, 0/20; low-dose, 1/49; high-dose, 7/48 ($p = 0.006$, test for linear trend); and of transitional- and squamous-cell carcinomas of the urinary bladder in females: control, 0/18; low-dose, 2/45; high-dose, 12/48 ($p < 0.001$, test for linear trend). Increases were also seen in the incidences of phaeochromocytomas of the adrenal gland in females: control, 1/20; low-dose, 0/50; high-dose, 14/48 ($p < 0.001$; χ^2 for 2 x 3 contingency table). The incidences of liver tumours, all considered to be neoplastic nodules [adenomas (Maronpot *et al.*, 1986)], were also increased in females: control, 2/15; low-dose, 8/49; high-dose, 22/49 ($p = 0.001$, test for linear trend) (National Cancer Institute, 1977).

NTA, trisodium salt, monohydrate

Mouse: Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were fed 2500 or 5000 (maximum tolerated dose) ppm (mg/kg) commercial-grade NTA, trisodium salt, monohydrate (purity, 99.5%) in the diet for 18 months and were killed at 21 months. Groups of 20 male and 20 female mice served as controls. A dose-related decrease in body weight gain was observed in mice of each sex; survival was comparable in treated and control animals. No urinary-tract tumour was observed, but there was a dose-related increase in the

incidence of haematopoietic tumours in male mice: control, 0/20; low-dose, 4/47; high-dose, 9/50 ($p = 0.015$, test for linear trend) (National Cancer Institute, 1977).

Rat: Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were fed diets containing 7500 or 15 000 (maximum tolerated dose) ppm (mg/kg) commercial-grade NTA, trisodium salt, monohydrate (purity, 99.5%) for 18 months and were killed at 24 months. Groups of 20 male and 20 female rats served as controls. A dose-related decrease in body weight was observed in rats of each sex; survival was comparable in treated and control animals. Evidence of renal inflammation was observed in low- and high-dose male and female rats, but no increase in the incidence of neoplasms was observed (National Cancer Institute, 1977).

In another feeding study, groups of 24 male and 24 female Fischer 344 rats, 51-55 days of age, were fed 0, 200, 2000 or 20 000 (maximum tolerated dose) ppm (mg/kg) commercial-grade NTA, trisodium salt, monohydrate (no impurity detected) in the diet for 104 weeks. Decreases in body weight and survival and hydronephrosis were observed in high-dose males. One papilloma of the urinary bladder was seen in a female given 2000 ppm; all other kidney and urinary-tract tumours were observed in males and females given the highest dose. Tubular-cell adenomas and adenocarcinomas of the kidney were observed in 4/24 males [$p = 0.004$, Cochran-Armitage test]; transitional-cell carcinomas developed in the renal pelvis in 4/24 males ($p = 0.003$, Cochran-Armitage test) and in the ureter in 8/24 males ($p < 0.001$, Cochran-Armitage test); and metastases were observed in 5/24 males. Tubular-cell adenomas and adenocarcinomas of the kidney developed in 4/24 females ($p = 0.003$, test for linear trend); transitional-cell carcinomas developed in the ureter in 6/24 females ($p < 0.001$, test for linear trend) and in the urinary bladder in 5/24 females ($p = 0.001$, test for linear trend); metastases were observed in 5/24 females (National Cancer Institute, 1977).

(*b*) *Administration in drinking-water*

NTA, trisodium salt

Rat: A total of 196 male, non-inbred Sprague-Dawley rats, weighing approximately 350 g, were given 0.1% NTA, trisodium salt [purity unspecified] in the drinking-water *ad libitum* for 704 days. A group of 192 untreated males served as controls. No difference in weight gain was observed between control and treated animals, but a higher proportion of treated rats died during the first 550 days of the study. An increase in the incidence of renal adenomas and adenocarcinomas was observed, with adenomas in 5/186 controls and 25 adenomas and four carcinomas in 29/183 treated animals ($p < 0.01$, Mantel-Haenszel test). In addition, renal tubular hyperplasia (grades III and IV) was observed 44/186 controls and 67/183 treated animals. There was no apparent difference in the frequency of severe nephritis between control and treated animals (Goyer *et al.*, 1981).

NTA, disodium salt

Mouse: Groups of 40 male and 40 female random-bred Swiss mice, eight weeks of age, were given 5 g/l NTA disodium salt, monohydrate [purity unspecified] in the drinking-water for 26 weeks and killed at 35-36 weeks. No increase in the incidence of tumours at any site

was observed (Greenblatt & Lijinsky, 1974). [The Working Group noted the short duration of the experiment and the relatively low dose used.]

Rat: Groups of 15 male and 15 female MRC rats, eight to ten weeks of age, were given approximately 20 ml of drinking-water containing 0.5% NTA, disodium salt [purity unspecified] on five days a week for 84 weeks. All surviving animals were killed 104 weeks after the beginning of treatment. No significant difference in tumour incidence and no toxicity were observed (Lijinsky *et al.*, 1973). [The Working Group noted the small number of animals and the relatively low dose used.]

(*c*) *Administration with known carcinogens*[1]

Rat: In a two-stage carcinogenicity study of NTA, trisodium salt, monohydrate, four groups of 21 male Wistar rats, seven weeks old, received 0.05% *N*-nitroso(4-hydroxybutyl)butylamine (NHBBA) [purity unspecified] in the drinking-water for four weeks, after which they were fed 0 (NHBBA alone), 0.3%, 0.5% or 1.0% NTA, trisodium salt, monohydrate (purity, >95%) in the diet for 28 weeks, when all survivors were killed. An increased incidence of papillary or nodular [transitional-cell] hyperplasia of the urinary bladder was observed with all three doses of NTA, trisodium salt, monohydrate: NHBBA alone, 3/20; low-dose, 13/21; mid-dose, 18/18; high-dose, 17/17 [$p < 0.001$, Cochran-Armitage test]. An increase was also detected in the incidence of [transitional-cell] papillomas of the urinary bladder: NHBBA alone, 0/20; low-dose, 1/21; mid-dose, 8/18; high-dose, 12/17 [$p < 0.001$, Cochran-Armitage test]. There was also an increased incidence of transitional-cell carcinomas of the urinary bladder: NHBBA alone, 0/20; low-dose, 1/21; mid-dose, 2/18; high-dose, 7/17 [$p < 0.001$, Cochran-Armitage test]. In three additional groups that received 0.3, 0.5 or 1.0% NTA, trisodium salt, monohydrate without NHBBA, simple [transitional-cell] hyperplasia of the urinary bladder was observed frequently (Kitahori *et al.*, 1985).

In a similar study, five groups of 25-26 male Fischer 344 rats were given 0, 0.01% or 0.05% NHBBA [purity unspecified] in the drinking-water for four weeks and were then fed diets containing 0 or 2% NTA, trisodium salt, monohydrate (95.0% pure) for 32 weeks, at which time survivors were killed. In animals treated with 0.05% NHBBA plus NTA, trisodium salt, monohydrate, an increased incidence of papillary or nodular [transitional-cell] hyperplasia of the urinary bladder was observed: NHBBA, 13/26; NHBBA plus NTA, trisodium salt, monohydrate, 23/26 ($p < 0.01$). [Transitional-cell] papillomas of the urinary bladder were also observed in 8/26 rats gien NHBBA alone and in 18/26 also given the NTA compound ($p < 0.01$). In animals treated with the NTA compound alone, no hyperplastic or neoplastic lesion was observed (Fukushima *et al.*, 1985). [The Working Group noted the short duration of the experiment and the lack of specification of the statistical test used.]

[1]The Working Group noted that the four studies described were designed as two-stage carcinogenicity studies and could therefore not be evaluated as complete carcinogenicity studies.

In a further two-stage carcinogenicity study, eight groups of 24 male inbred Wistar rats, seven weeks of age, were fed a diet containing 0 or 1000 ppm (mg/kg) *N*-nitrosoethylhydroxyethylamine (NEHEA) (purity, 99.8%) for two weeks after which they were given 0, 3000, 10 000 or 30 000 ppm NTA, trisodium salt, monohydrate (95.0% pure) in the diet for 30 weeks, at which time survivors were killed. A significant increase in the incidence of renal tubular-cell tumours was observed in animals treated with NEHEA plus the mid and high doses of the NTA compound over that in animals treated with NEHEA alone: NEHEA alone, 4/24; low-dose, 5/22; mid-dose, 23/23 ($p < 0.01$); and high-dose, 23/23 ($p < 0.01$). In animals treated with the NTA compound alone, no renal tubular-cell tumour was observed (Hiasa *et al.*, 1985). [The Working Group noted the short duration of the experiment.]

In a two-stage carcinogenicity study, groups of 15-20 male Wistar rats, weighing 130-150 g, were given drinking-water containing 0.2% *N*-nitrosobis(2-hydroxypropyl)amine (NDHPA; purity, 98%) for two weeks and were then fed 1% NTA (purity, 99%) or its trisodium salt, monohydrate (purity, 95%) in the diet for 30 weeks. The tumour incidences in the groups treated with NDHPA, with NDHPA plus NTA and with NDHPA plus NTA, trisodium salt, monohydrate were: urinary-bladder tumours (mainly papillomas)—1/20, 1/20 and 7/20 [$p = 0.004$, Cochran-Armitage test]; renal cell tumours—3/20, 15/20 and 10/20 [$p = 0.014$, Cochran-Armitage test]; and nephroblastomas—3/20, 4/20 and 11/20 [$p = 0.003$, Cochran-Armitage test] (Shimoyama, 1986).

Solutions of NTA, disodium salt with metal salts

Intraperitoneal injection

Mouse: A group of 53 male and 21 female A/J mice, four weeks old, received daily intraperitoneal injections of solutions prepared from ferric nitrate and NTA, disodium salt (molar ratio of iron to NTA, 1:4; Fe-NTA), adjusted to pH 7.0 with Na_2HCO_3 at a level of 1.8-2.7 mg/kg bw iron, on six days per week for 12 weeks. The surviving animals were killed at 420 days. A further group of ten males and ten females was injected with equivalent amounts of NTA (reagent grade [purity unspecified]), and 20 males and 20 females were untreated. Fe-NTA was highly toxic to males: 28/53 died within 14 days, mainly from renal failure as a consequence of proximal tubular necrosis; no lethality was observed in females. Of the animals that survived to 420 days, 15/25 Fe-NTA-treated males ($p < 0.005$, χ^2 test) and 1/21 Fe-NTA-treated females developed renal tubular-cell adenocarcinomas. The first tumours appeared at 50 days. Renal neoplasms did not occur in untreated controls or in mice treated only with NTA (Li *et al.*, 1987). [The Working Group noted the short duration of the experiment.]

Rat: A group of 32 male Wistar rats [age unspecified] received six intraperitoneal injections per week of solutions of ferric nitrate and NTA, disodium salt, as described above, for two weeks (total dose, 100-150 mg iron per animal) and were kept for 240-260 days. Eight male rats served as untreated controls and eight as saline-treated controls. Eight animals in the treated group died during the study; serial sacrifices were made throughout the experiment. At termination of the study, 22/24 Fe-NTA-treated rats but no saline-treated or untreated control had developed renal adenocarcinomas (Okada & Midorikawa, 1982). [The Working Group noted the small number of animals used and the short duration of the study.]

Groups of 24 male Wistar rats, four weeks of age, received daily intraperitoneal injections of solutions prepared from ferric nitrate and NTA, disodium salt, as described above (5-7 mg iron/kg bw per day) or solutions prepared from aluminium chloride and NTA, disodium salt (Al-NTA; molar ratio of aluminium to NTA, 1:4; 1.5-2.0 mg aluminium/kg bw per day) on six days a week for up to three months and were killed at 52 weeks. Groups of ten males received injections of equivalent amounts of saline, solutions of NTA or solutions of aluminium chloride. Rats treated with Al-NTA or Fe-NTA had depressed weight gain and developed severe injury of the proximal convoluted tubules, polyuria and glucosuria. Fe-NTA-treated rats developed renal-cell carcinomas (14/18; $p < 0.05$); no such tumour was observed in the other groups (Ebina *et al.*, 1986). [The Working Group noted the small number of animals used and the short duration of the study.]

3.2 Other relevant biological data

(*a*) *Experimental systems*

(i) *Absorption, distribution, excretion and metabolism*

NTA and its salts

The absorption, distribution and excretion of NTA, trisodium salt have been reviewed (Anderson *et al.*, 1985). Absorption of NTA, disodium salt in rats and dogs ranged from 77% to 99% after single oral doses (Michael & Wakim, 1971). In a whole-body autoradiographic study, 0.93 mg [1-^{14}C-acetate]-NTA, trisodium salt was administered intravenously to NMRI albino mice and the same amount orally to C57Bl mice; heavy accumulation of radioactivity occurred in the skeleton, which persisted for 48 h, the longest interval studied (Tjälve, 1972).

The kidney attains concentrations of NTA greater than that in the plasma in rats with steady-state plasma NTA levels. The relatively high kidney concentrations of NTA can be attributed to high concentrations of NTA in small volumes of urine (Anderson, 1980).

In rats and dogs, the only route of excretion of absorbed NTA, disodium salt was *via* the urine, as shown by the absence of ingested NTA in the bile; in monkeys and rabbits, it was excreted in the faeces (Michael & Wakim, 1971).

NTA is not metabolized in mammals and is excreted rapidly by filtration in the kidney (Michael & Wakim, 1971; Budny, 1972; Chu *et al.*, 1978; Anderson *et al.*, 1985).

Solutions of NTA, disodium salt with metal salts

Repeated intraperitoneal injections of a solution of ferric nitrate and NTA, disodium salt (molar ratio, 2:1) resulted in deposition of iron in the parenchymal cells of the liver and in the pancreas and adrenal glands of rats and rabbits (Awai *et al.*, 1979). Iron uptake by rat liver was examined after a single intraperitoneal injection of ^{59}Fe-NTA (Fe:NTA, 1:5 molar ratio) and of Fe-[^{14}C]-NTA (Fe:NTA, 1:1; prepared as above) to give 7.5 mg Fe/kg bw. Of the injected ^{59}Fe, 30% was incorporated in the liver non-haem iron fraction by 3 h and was retained for 240 h; only 1% of the ^{14}C injected as Fe-[^{14}C]-NTA was taken up by the liver by 3 h (Matsuura, 1983).

(ii) *Toxic effects*

NTA and its salts

The toxicology of NTA has been reviewed (Anderson *et al.*, 1985).

The oral LD_{50} in rodents of NTA, trisodium salt, monohydrate was reported to be about 2 g/kg bw (Anderson *et al.*, 1985).

NTA is nephrotoxic. NTA and its trisodium salt have been shown to induce cytoplasmic vacuolation of renal proximal tubule cells, hydronephrosis, erosion and ulceration of the renal pelvic transitional epithelium and kidney hyperplasia in rats at dietary levels of 0.15% or more, in studies of 28 days or longer (Nixon, 1971; Mahaffey & Goyer, 1972; Nixon *et al.*, 1972; National Cancer Institute, 1977; Alden *et al.*, 1981; Merski, 1981; Alden & Kanerva, 1982a; Anderson *et al.*, 1982; Merski, 1982) and in mice at 0.5% or more (National Cancer Institute, 1977). In a comparison study in treated and untreated animals, all forms of renal toxicity, except hydronephrosis, basophilic hyperplasia of tubular cells and neoplasia, were reversed when treatment was discontinued (Alden & Kanerva, 1982b; Myers *et al.*, 1982). Ureters were swollen and showed alterations in epithelial morphology similar to those observed in the renal pelvis (Kanerva *et al.*, 1984).

The responses of Charles River and Fischer 344 rats to 1.5% NTA and 2% NTA, trisodium salt in the diet were evaluated in a four-week feeding study. In spite of different rates of ingestion, the two strains of animals had similar qualitative responses to NTA. Ingestion of NTA or its trisodium salt was associated with reduced growth, increased kidney:body weight ratio, increased urinary calcium, haematuria and the presence of crystalline NTA, calcium sodium salt in the urine (Anderson & Kanerva, 1979). Feeding a dose of 1.5% NTA (which induced bladder neoplasms) to rats was associated with a 50% decrease in the calcium concentration of bladder tissue, but with little change in magnesium, zinc, sodium or potassium levels (Anderson *et al.*, 1982).

No change in fat-free bone weight, total ash or percentage of ash was found in the tibias of rats fed NTA, trisodium salt for 91 days. When given for 30 days, the compound had no effect on serum alkaline phosphatase or on liver and kidney carbonic anhydrase (Michael & Wakim, 1973), but hepatic metallothionein levels were increased two fold following intraperitoneal administration of NTA (100 mg/kg bw) to male Swiss Webster mice (Goering *et al.*, 1985).

After NTA, trisodium salt was fed to male and female beagle dogs for 90 days at 0.03, 0.15 and 0.5% in the diet, urinary zinc excretion was significantly greater in dogs in the mid- and high-dose groups. NTA was deposited in bone (123-142 ppm at the 0.5% dose level), but this had no adverse effect (Budny *et al.*, 1973).

Solutions of NTA with metal salts

In the studies described below, animals were given solutions prepared from a ferric salt (usually ferric nitrate) and an NTA salt (usually NTA, disodium salt) in various molar ratios, as specified. In all cases, the solution contained an excess of either NTA or iron, which would be present in addition to the Fe-NTA complex in solution. The precise composition of the solutions was not further characterized by the authors.

Twenty-four hours after a single intraperitoneal injection of Fe-NTA (Fe:NTA, 1:4 molar ratio) to Wistar rats (Hamazaki *et al.*, 1985; Ebina *et al.*, 1986) or A/J mice (Li *et al.*, 1987), rats developed extensive necrosis of the renal proximal tubules, which progressed with multiple doses to partial renal degeneration, strictly confined to the proximal tubules. Sequalae were polyuria, glucosuria and aminoaciduria. In mice, the predominant autopsy finding among those (all males) that died within 14 days was renal tubular necrosis. Vitamin E protected Wistar rats against the nephrotoxic effects of Fe-NTA (Fe:NTA, 1:4—Okada *et al.*, 1987; Fe:NTA, unspecified—Hamazaki *et al.*, 1988). Mild diabetes has been reported after daily injection of Fe-NTA in rats and rabbits (Fe:NTA, 2:1—Awai *et al.*, 1979; Fe:NTA, 1:2-2.5—May *et al.*, 1980). Fe-NTA is a potent initiator of lipid peroxidation in the livers of rats (Fe:NTA, 1:1.5; Goddard *et al.*, 1986) and mice (Fe:NTA, 1:1.5; Goddard & Sweeney, 1983) injected intraperitoneally, in rat liver homogenates and hepatocyte suspensions (Fe:NTA, 1:1.5; Goddard *et al.*, 1986) and in Ehrlich ascites tumour cells (Fe:NTA, 1:5; Nakamoto *et al.*, 1986). It was reported in an abstract that oral administration of Fe-NTA [molar ratio unspecified] to male Fischer 344 rats increased the formation of 8-hydroxydeoxyguanosine in the kidney (Sai *et al.*, 1988).

Male Wistar rats given solutions prepared from aluminium chloride and NTA disodium salt (Al:NTA, 1:4; 1.5-2.0 mg Al/kg bw per day) by intraperitoneal injection for 14 days showed morphological damage in the liver and kidney, including diffuse mid-zonal coagulation necrosis of hepatocytes and acute proximal tubular necrosis of the kidney at day 4. Seven of ten rats given Al-NTA died within five days. When Al-NTA was given in a dose of 1.5-2.0 mg Al/kg bw per day for 54 days, metabolic acidosis was demonstrated and renal injury was severe, involving proximal tubular necrosis and granular casts in the distal tubules. From day 38 onwards, atrophy of the nerve cells of the cerebrum and demyelination of the brain stem were also observed (Ebina *et al.*, 1984, 1986).

(iii) *Effects on reproduction and prenatal toxicity*

Ten NMRI mice received 0.2% NTA in the drinking-water on days 6-18 of gestation; ten control mice received no treatment. A small difference in mean fetal weight was seen between the two groups on day 18, and the number of resorptions was slightly higher in the treated group. Skeletal and visceral examination did not reveal any teratogenic effect (Tjälve, 1972).

Groups of 20 mated female Charles River rats were given 0, 0.1 or 20 mg/kg bw NTA, trisodium salt, monohydrate (two groups for each dose) in the drinking-water on days 6-14 of gestation. Treatment did not affect the numbers of resorptions or fetuses, fetal body weight or development of the fetal skeleton. A significant increase in the incidence of hydronephrosis and bladder defects was observed in fetuses in some treated groups (Nolen *et al.*, 1972a).

NTA, trisodium salt did not induce reproductive toxicity in male or female Charles River CD rats in a two-generation study (0.1 or 0.5% in the diet) or in pregnant Charles River CD rats (0.1 or 0.5% in the diet on days 6-15 of pregnancy) or in rabbits (up to 250 mg/kg bw by gavage on days 7-16 of gestation; Nolen *et al.*, 1971).

The available studies provide no evidence that NTA or its salts enhance the reproductive toxicity of heavy metals in experimental animals (Nolen *et al.*, 1972a,b; Scharpf *et al.*, 1972, 1973; McClain & Siekierka, 1975).

(iv) *Genetic and related effects* (see Appendix 1)

NTA and its salts

NTA at concentrations up to 42.5 mM did not induce forward mutation or aneuploidy in *Aspergillus nidulans* (Crebelli *et al.*, 1986). It did not induce respiration-defective mutants in *Saccharomyces cerevisiae* at a concentration of 4 g/l (Zetterberg, 1970), not did it induce sex-linked recessive lethal mutations in *Drosophila* in feeding and injection studies (Kramers, 1976—50 mM and 10 mM, respectively; Woodruff *et al.*, 1985—4000 and 1000 ppm, respectively); however, it did induce aneuploidy (at 5×10^{-2}M; Costa *et al.*, 1988a) and sex-chromosome loss (at 4000 ppm, Ramel & Magnusson, 1979) in *Drosophila*. NTA (0.5-5 μg/ml) did not induce sister chromatid exchange or chromosomal aberrations in the Chinese hamster CHO cell line in culture in the presence or absence of an exogenous metabolic system from Aroclor 1254-induced rat liver (Loveday *et al.*, 1989). It was reported in an abstract to induce ploidy changes and endoreduplication in human lymphocytes *in vitro* (Bora, 1975). NTA did not cause dominant lethal mutation in mice *in vivo* at 125 mg/kg bw given intraperitoneally or 1000 mg/kg bw given orally (Epstein *et al.*, 1972).

NTA, trisodium salt was not mutagenic to several strains of *Salmonella typhimurium* (Dunkel *et al.*, 1985; Loprieno *et al.*, 1985; Venier *et al.*, 1987) or to *Escherichia coli* (Dunkel *et al.*, 1985; Venier *et al.*, 1987) in the presence or absence of an exogenous metabolic system from Aroclor 1254-induced rat liver (Dunkel *et al.*, 1985; Loprieno *et al.*, 1985; Venier *et al.*, 1987), mouse liver or Syrian hamster liver or uninduced rat, mouse or Syrian hamster liver (Dunkel *et al.*, 1985). It was not active in the SOS chromotest but gave positive results at a dose of 1 mg in differential toxicity tests in *E. coli* in the presence and absence of an exogenous metabolic system (Venier *et al.*, 1987).

NTA, trisodium salt induced chromosomal aberrations at 2×10^{-2}M in *Vicia faba* (Kihlman & Sturelid, 1970); it induced micronuclei in *V. faba* at 2×10^{-3}M and in *Allium cepa* at 4×10^{-3}M (De Marco *et al.*, 1986). NTA, trisodium salt was not mutagenic to *Saccharomyces pombe* or *Saccharomyces cerevisiae* (40 μg/ml) in the presence or absence of an exogenous metabolic system (Loprieno *et al.*, 1985). The trisodium salt did not cause unscheduled DNA synthesis in primary cultures of rat hepatocytes at 0.5 or 1 mg/ml (Williams *et al.*, 1982). It did not cause mutation at the *hprt* locus in Chinese hamster V79 cells *in vitro* at 10^{-2}M (Celotti *et al.*, 1987) and did not induce mutation at the TK locus in mouse lymphoma L5178Y cells *in vitro* in the presence or absence of an exogenous metabolic sytstem (630-2350 μg/ml or 524-1900 μg/ml, respectively; Mitchell *et al.*, 1988). It did not induce sister chromatid exchange in the Chinese hamster CHO cell line (10^{-3}M, Ved Brat & Williams, 1984; 1.9 μg/ml, Loprieno *et al.*, 1985; 2×10^{-3}M, Montaldi *et al.*, 1985; 1 μg/ml, Venier *et al.*, 1985) or in cultured peripheral lymphocytes from Balb/c and Balb/Mo mice (10^{-3}M; Montaldi *et al.*, 1985). It induced chromosomal aberrations in rat kangaroo cells *in vitro* at 2×10^{-2}M (Kihlman & Sturelid, 1970), and it induced resistance to diphtheria toxin in a human epithelial-like cell line *in vitro* (1.1×10^{-5}M; Grilli & Capucci, 1985). It did not induce sister chromatid ex-

change (10^{-3}M; Ved Brat & Williams, 1984) or chromosomal aberrations (10^{-2}M, Ved Brat & Williams, 1984; 7.5×10^{-3}M, Montaldi *et al.*, 1988) in cultured human peripheral lymphocytes. It did not induce micronuclei in mice *in vivo* (200-400 mg/kg bw; Montaldi *et al.*, 1988).

As reported in an abstract, NTA, sodium calcium salt did not induce heritable translocations in mice *in vivo* after administration of 0.1% in drinking-water for seven weeks (Jorgenson *et al.*, 1975).

Solutions of NTA with metal salts

Lead chromate ($PbCrO_4$) was mutagenic to *S. typhimurium* (Loprieno *et al.*, 1985; Venier *et al.*, 1987) and to *E. coli* (Venier *et al.*, 1987) in the presence of NTA, trisodium salt, but not when tested alone. Similarly, lead chromate in a NTA, trisodium salt solution induced mutation in *Drosophila* (Costa *et al.*, 1988) and mutations at the *hprt* locus in Chinese hamster V79 cells (Celotti *et al.*, 1987). NTA, trisodium salt (2×10^{-3}M-6×10^{-3}M) enhanced the frequency of sister chromatid exchange in the Chinese hamster CHO cell line induced by the insoluble salts $CdCO_3$, HgCl, $PbSO_4$, $PbCrO_4$ and $NiCO_3$ (Loprieno *et al.*, 1985; Montaldi *et al.*, 1987), as well as that induced by insoluble chromates of Ba, Zn, Sr and Ca (Venier *et al.*, 1985); no such enhancement was seen for soluble salts ($CdCl_2$, $K_2Cr_2O_7$, $HgCl_2$ and $NiCl_2$; Montaldi *et al.*, 1987). Solutions of NTA:iron (2:1 molar ratio), with the iron as $FeSO_4$ and $FeCl_3$, caused chromosomal aberrations in the Chinese hamster CHO cell line only at millimolar concentrations (Whiting *et al.*, 1981). NTA, trisodium salt (2×10^{-3}M) enhanced the frequency of micronuclei and chromosomal aberrations induced in human lymphocytes by the insoluble salts $CdCO_3$, HgCl and $PbSO_4$, and, to a lesser extent, that induced by $PbCrO_4$ and $NiCO_3$ (Montaldi *et al.*, 1987). Transformation of Syrian hamster fibroblast BHK cells induced by Cr[VI] compounds was enhanced by the presence of NTA (Lanfranchi *et al.*, 1988).

[The Working Group noted that when NTA or its salts and NTA in combination with metal salts caused a positive response, primarily in assays for chromosomal anomalies, the effects were seen only with very high concentrations of NTA.]

(*b*) *Humans*

(i) *Absorption, distribution, excretion and metabolism*

A capsule containing 10 mg [1-^{14}C]NTA in gelatin was given orally in fruit juice to each of eight male volunteers who had received no drugs for two weeks before entering the study. Twelve percent of the administered radioactivity was excreted in the urine and 77% in the faeces as unchanged NTA within 120 h of administration. A peak in the blood concentration (6.5 ng/g serum) occurred 1-2 h after dosing (Budny & Arnold, 1973).

(ii) *Toxic effects*

No data were available to the Working Group.

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Genetic and related effects*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Nitrilotriacetic acid and its sodium salts have been produced since the 1930s for use as metal chelating agents in household and industrial detergents, industrial water treatment, textile preparation and metal finishing. Occupational exposure to nitrilotriacetic acid and its salts may occur during its production and use, but data on levels are limited. Exposure to nitrilotriacetic acid, and presumably to its water-soluble metal complexes, occurs as a result of its presence in household detergents and in drinking-water.

4.2 Experimental carcinogenicity data

Nitrilotriacetic acid was tested for carcinogenicity by oral administration in the diet in mice and rats. It induced renal-cell adenocarcinomas in mice of each sex, renal-cell tumours in male rats and transitional- and squamous-cell carcinomas of the urinary bladder, hepatocellular adenomas and adrenal phaeochromocytomas in female rats.

Nitrilotriacetic acid, trisodium salt was tested for carcinogenicity in mice and rats by oral administration. When administered in the diet as the monohydrate, it induced haematopoietic tumours in male mice and benign and malignant tumours of the urinary system (kidney, ureter and bladder) in rats of each sex. When administered in drinking-water to male rats, it induced renal adenomas and adenocarcinomas.

In two-stage carcinogenicity studies in male rats by oral administration, nitrilotriacetic acid and its trisodium salt increased the incidence of urinary-tract tumours after pretreatment with different nitrosamines.

Solutions of nitrilotriacetic acid, disodium salt with ferric salts were tested in mice of each sex and in male rats by intraperitoneal administration. They induced renal adenocarcinomas in males of each species.

4.3 Human carcinogenicity data

No data were available to the Working Group.

4.4 Other relevant data

Nitrilotriacetic acid and its trisodium salt were nephrotoxic to rodents.

In a single study, nitrilotriacetic acid did not induce dominant lethal mutation in mice treated *in vivo*. Also in single studies, it did not induce chromosomal aberrations or sister

chromatid exchange in Chinese hamster cells *in vitro*. In single studies, it induced aneuploidy and sex chromosome loss in *Drosophila* at high doses. In other studies, it did not induce sex-linked recessive lethal mutation in *Drosophila*. It was not mutagenic to fungi, and, in a single study, it did not cause aneuploidy in fungi.

In a single study, nitrilotriacetic acid, trisodium salt did not induce micronuclei in mice *in vivo*. It did not cause chromosomal aberrations or, in a single study, sister chromatid exchange in human peripheral lymphocytes *in vitro*, but, at a high dose in one study, it was mutagenic to a human epithelial-like cell line *in vitro*. It also caused chromosomal aberrations in rat kangaroo cells at a high dose in a single study, but it did not cause sister chromatid exchange or gene mutation or, in another study, unscheduled DNA synthesis in rodent cells *in vitro*. At high doses, it caused chromosomal aberrations in plants. It was not mutagenic to yeast or bacteria in the presence or absence of an exogenous metabolic system. It gave equivocal results for DNA damage in prokaryotes.

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity of nitrilotriacetic acid and its sodium salts in experimental animals.

No data were available from studies in humans on the carcinogenicity of nitrilotriacetic acid and its salts.

In formulating the overall evaluation, the Working Group took note of the fact that nitrilotriacetic acid is liberated to some extent from nitrilotriacetate salts in solution.

Overall evaluation

Nitrilotriacetic acid and its salts are *possibly carcinogenic to humans (Group 2B)*.

5. References

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[1]For description of the italicized terms and criteria for making the evaluation, see Preamble, pp. 25-29.

Summary table of genetic and related effects of nitrilotriacetic acid

Nonmammalian systems													**Mammalian systems**																													
Prokaryotes		Lower eukaryotes				Plants			Insects				*In vitro*																*In vivo*													
													Animal cells								Human cells								Animals							Humans					
D	G	D	R	G	A	D	G	C	R	G	C	A	D	G	S	M	C	A	T	I	D	G	S	M	C	A	T	I	D	G	S	M	C	DL	A	D	S	M	C	A
				-	-[1]					-	+[1]	+[1]			-[1]		-[1]																		-[1]							

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

In completing the tables, the following symbols indicate the consensus of the Working Group with regard to the results for each endpoint:

- \- considered to be negative
- -[1] considered to be negative, but only one valid study was available to the Working Group
- +[1] considered to be positive, but only one valid study was available to the Working Group

Summary table of genetic and related effects of nitrilotriacetic acid, trisodium salt

Nonmammalian systems													**Mammalian systems**																											
Proka-ryotes		Lower eukaryotes				Plants			Insects				*In vitro*												*In vivo*														
													Animal cells								Human cells								Animals							Humans				
D	G	D	R	G	A	D	G	C	R	G	C	A	D	G	S	M	C	A	T	I	D	G	S	M	C	A	T	I	D	G	S	M	C	DL	A	D	S	M	C	A
?	–			–				+					–[1]	–[1]	–		+[1]					+	–[1]		–							–[1]								

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

In completing the tables, the following symbols indicate the consensus of the Working Group with regard to the results for each endpoint:

? considered to be equivocal or inconclusive (e.g., there were contradictory results from different laboratories; there were confounding exposures; the results were equivocal)

– considered to be negative

\+ considered to be positive for the specific endpoint and level of biological complexity

–[1] considered to be negative, but only one valid study was available to the Working Group

+[1] considered to be positive, but only one valid study was available to the Working Group

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