Screening Assessment for the Challenge

Glycine, *N*,*N*-bis(carboxymethyl)- (Nitrilotriacetic acid)

Chemical Abstracts Service Registry Number
139-13-9

Environment Canada
Health Canada

July 2010

Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment of glycine, *N*,*N*-bis(carboxymethyl)-, commonly referred to as nitrilotriacetic acid or NTA, Chemical Abstracts Service Registry Number 139-13-9. This substance was identified in the categorization of the *Domestic Substances List* as a high priority for action under the Challenge. NTA was identified as a high priority as it was determined to present intermediate potential for exposure of individuals in Canada and had been classified by other agencies on the basis of carcinogenicity. Although it was concluded that NTA met the ecological categorization criteria for persistence, it did not meet the criteria for bioaccumulation potential or inherent toxicity to aquatic organisms. Therefore, the focus of this assessment of NTA relates primarily to human health risks. Information relating to the sodium salts of NTA is also considered in this screening assessment because the toxicological endpoints of NTA and its sodium salts are similar and because the dissociation of NTA and its sodium salts leads to a common moiety. However, the conclusion of this screening assessment pertains to NTA only; the data presented relating to the sodium salts of NTA serve only as supporting information.

According to information reported in response to a notice published under section 71 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), no companies in Canada reported manufacturing NTA in a quantity greater than or equal to the reporting threshold of 100 kg for the 2006 calendar year. However, it was reported that NTA was imported into Canada in the range of 1000–10 000 kg in that year. The total quantity of NTA (including its salts) imported in 2006, as reported by another source, was 3.9 million kilograms. Available scientific and technical information and responses to the section 71 survey indicated that the major use of NTA in Canada is in institutional and industrial cleaning products. Other applications of NTA in Canada involve its function as a chelating agent in a variety of industrial processes, such as the industrial treatment of boiler water and pulp and paper processing to produce paper and paperboard products. Previously, the trisodium salt of NTA (Na_3NTA) was used heavily as a builder in laundry detergents. Currently, the major use of this salt in Canada lies also in institutional and industrial applications. Na_3NTA is a non-active ingredient in Canadian pesticide formulations and has been found in a limited number of Canadian personal care products.

Emissions of NTA and its salts into the ambient environment are expected to be primarily from anthropogenic activities. NTA and its salts have been measured in Canadian municipally treated drinking water, groundwater and industrial water. Exposures of the general population to NTA via intake of drinking water and use of consumer products are estimated to be low.

High concentrations of NTA induced primary tumours at several locations in the urinary tract after administration in the diet or in drinking water in long-term rat and mouse studies. Transitional cell epithelial tumours were observed in the kidney, ureter and urinary bladder, while tubular cell tumours were observed in the kidney. In short-term to chronic repeated-dose studies in experimental animals exposed orally to NTA, non-

neoplastic lesions were often observed in the kidney, ureters and urinary bladder. Consideration of the available information regarding genotoxicity indicates that NTA is not likely to be genotoxic. Accordingly, although the mode of induction of tumours is not fully elucidated, the tumours observed are not considered to have resulted from direct interaction with genetic material. Therefore, a threshold approach is used to assess risk to human health.

Based on the comparison of estimated exposures to NTA in Canada with the critical effect levels, and taking into account the uncertainties in the databases related to exposure and hazard characterization, it is considered that the resulting margins of exposure are adequately protective of human health.

Based on the available information on its potential to cause harm to human health, it is concluded that NTA is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

An ecological exposure scenario was developed based on the information on commercial use to conservatively estimate releases into the aquatic environment from industrial operations and resulting aquatic concentrations. Environmental concentrations are estimated to be below those that would harm sensitive aquatic organisms. This indicates that the substance is unlikely to cause ecological harm in the aquatic environment. In addition, information was identified indicating that NTA is likely to degrade quickly in environmental media.

Based on the information available, it is concluded that NTA is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. NTA does not meet the persistence or bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*.

Based on the information available, it is concluded that NTA does not meet any of the criteria set out in section 64 of CEPA 1999.

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006), which challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance nitrilotriacetic acid (NTA) was identified as a high priority for assessment of human health risk because it was considered to present IPE and had been classified by other agencies on the basis of carcinogenicity.

The Challenge for NTA was published in the *Canada Gazette* on January 31, 2009 (Canada 2009). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information were received.

Although NTA was determined to be a high priority for assessment with respect to human health and also met the ecological categorization criteria for persistence, it did not meet the criteria for bioaccumulation potential or inherent toxicity to aquatic organisms. Therefore, this assessment focuses principally on information relevant to the evaluation of risks to human health.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA 1999. Screening assessments examine scientific information and develop conclusions by incorporating a weight of evidence approach and precaution.[1]

This final screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents and stakeholder research reports and from recent literature searches, up to April 2009 for the human health and ecological sections. Key studies were critically evaluated; modelling results may have been used to reach conclusions. Evaluation of risk to human health involves consideration of data relevant to estimation of exposure (non-occupational) of the general population, as well as information on health hazards (based principally on the weight of evidence assessments of other agencies that were used for prioritizing the substance). Decisions for human health are based on the nature of the critical effect and/or margins between conservative effect levels and estimates of exposure, taking into account confidence in the completeness of the identified databases on both exposure and effects, within a screening context. The screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents a summary of the critical information upon which the conclusion is based.

This final screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external written peer review/consultation. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (TERA), including Dr. Bernard Gadagbui (TERA), Dr. Michael Jayjock (The LifeLine Group) and Dr. Chris Bevans (CJB Consulting). Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment Canada. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel.

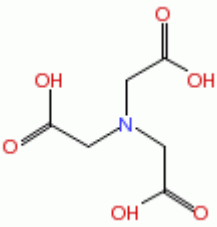
The critical information and considerations upon which the final screening assessment is based are summarized below.

[1] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA 1999 on the substances in the Chemicals Management Plan (CMP) Challenge Batches 1-12 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Controlled Products Regulations*, which is part of regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace use.

Substance Identity

For the purposes of this document, glycine, *N*,*N*-bis(carboxymethyl)- will be referred to as NTA, derived from the common name nitrilotriacetic acid. Information on the identity of NTA is summarized in Table 1.

Table 1. Substance identity

CAS RN	139-13-9
DSL name	Glycine, *N*,*N*-bis(carboxymethyl)-
NCI names	Glycine, *N*,*N*-bis(carboxymethyl)- (TSCA) Nitrilotriacetic acid (EINECS)
Other names	Aminotriacetic acid H_3NTA Nitrilotris(methylenecarboxylic acid) NTA NTA acid Triglycine
Chemical group (DSL stream)	Discrete organics
Major chemical subclass	Acids; tertiary amines
Chemical formula	$C_6H_9NO_6$
Chemical structure	
SMILES	O=C(O)CN(CC(=O)O)CC(=O)O
Molecular mass	191.14 g/mol

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; EINECS, European Inventory of Existing Commercial Chemical Substances; NCI, National Chemical Inventories; SMILES, simplified molecular input line entry specification; TSCA, *Toxic Substances Control Act* Chemical Substance Inventory.
Source: National Chemical Inventories (2008)

In this assessment, the evaluation of NTA was supplemented by information on the trisodium salt of NTA, commonly known as trisodium nitrilotriacetate (CAS RN 5064-31-3). This substance also exists in a monohydrate form, commonly known as trisodium nitrilotriacetate monohydrate (CAS RN 18662-53-8). Both of these trisodium salts will be referred to as Na_3NTA in this assessment. While this assessment includes supplementary information on Na_3NTA, only NTA is evaluated against the criteria outlined in section 64 of CEPA 1999.

Physical and Chemical Properties

Table 2 contains experimental and estimated physical and chemical properties of NTA that are relevant to its environmental fate. Key studies from which experimental data were reported for some of these properties were critically reviewed for validity. Models based on quantitative structure–activity relationships (QSARs) were used to generate data for some of the physical and chemical properties of NTA. These models are mainly based on fragment addition methods (i.e., they rely on the structure of a chemical). Since these models accept only the neutral (i.e., un-ionized) form of a chemical as input (in SMILES form), the modelled values shown in Table 2 are for the neutral form of NTA.

Table 2. Physical and chemical properties of NTA

Property	Type	Value	Temperature (°C)	Reference
Physical state	Undissociated acid form	Needles or prismatic crystals		WHO 1996
Melting point (ºC)	Experimental	242		PhysProp 2006
	Modelled	296.63		MPBPWIN 2008
Boiling point (ºC)	Modelled	429.28		MPBPWIN 2008
		498.2		ACD/Labs 2008
Density (kg/m^3)	Modelled	1610	20	ACD/Labs 2008
Vapour pressure (Pa)	Modelled	9.54×10^{-7}	25	MPBPWIN 2008
		3.7×10^{-9}	25	ACD/Labs 2008
Henry's Law constant (Pa·m^3/mol)	Modelled	1.21×10^{-11}	25	HENRYWIN 2008
Log K_{ow} (dimensionless)	Modelled	−3.81	25	KOWWIN 2008
		−1.764	25	ACD/Labs 2008
Log K_{oc} (dimensionless)	Modelled	−2.02		KOCWIN 2008
Log K_{oa} (dimensionless)	Modelled	10.5		KOAWIN 2008
Water solubility (mg/L)	Experimental	59 100	25	Yalkowsky and Dannenfelser 1992
	Modelled	249 060	25	WATERNT 2008
		1 000 000	25	ACD/Labs 2008

Property	Type	Value	Temperature (°C)	Reference
pK_a (dimensionless)	Modelled	pK_{a1} = 10.3 (most basic) pK_{a2} = 3.05 pK_{a3} = 2.27 pK_{a4} = 1.49 (most acidic)	25	ACD/pK_aDB 2005

Abbreviations: K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant.

NTA is a white solid at ambient temperature and pressure.

The modelling program pK_aDB from ACD/pK_aDB (2005) predicts that NTA ionizes in water in four steps: as a base in the first step, by attracting one proton to the tertiary amine group; and as an acid in the second, third and fourth steps, by giving up a proton from each of the three hydroxyl groups. There are therefore four pK_a values, as presented in Table 2.

With increasing pH, the extent of ionisation of NTA increases. In ambient waters negatively charged NTA molecules readily form complexes with the positively charged metal ions in solution (EURAR 2005).

Sources

NTA is an anthropogenic substance and does not occur naturally in the environment. It is present in the environment primarily as a result of its release in sewage from processing, use and disposal activities (Health Canada 1990). NTA is obtained through acidification of Na_3NTA at pH 1–2 (Gousetis and Opgenorth 2005). Traditionally, industrial production of Na_3NTA followed an alkaline process that formed a number of by-products (Coker 1972). Consequently, an acid process was later developed and is the method currently used by major manufacturers. In the acid process, ammonia is reacted with formaldehyde to form hexamethylenetetramine, which is subsequently treated with hydrogen cyanide in sulfuric acid to yield triscyanomethylamine. The cyano-intermediate is then saponified with sodium hydroxide to form Na_3NTA (Gousetis and Opgenorth 2005). Worldwide, the two leading manufacturers of NTA and its salts are BASF in Germany and Solutia in the United States (Suresh and Kishi 2006). Na_3NTA is the predominant form sold in Canada (Glauser et al. 2007).

Recent information was collected through industry surveys conducted for the year 2006 under a *Canada Gazette* notice issued pursuant to section 71 of CEPA 1999 (Canada 2009). This notice requested data on the manufacture, import and use quantities of NTA in Canada.

According to data submitted in response to the notice, no companies in Canada reported manufacturing NTA in a quantity greater than or equal to the reporting threshold of 100 kg for the 2006 calendar year. However, it was reported that this substance was imported into Canada in the range of 1000–10 000 kg in the same year (Environment Canada 2009a). The total quantity of NTA (including its salts) imported into Canada in 2006, as reported by another source, was 3.9 million kilograms (Glauser et al. 2007).

Uses

According to data submitted under section 71 of CEPA 1999 and other publicly available sources, NTA is reported to be used in Canada in institutional and industrial cleaning products, vehicle cleaners, asphalt paving, fertilizers, photographic developer solutions and descaling products for oil extraction and mining activities (Environment Canada 2009a). According to information in the available literature, institutional and industrial cleaning products constitute the largest Canadian application of NTA; these include general cleaners, degreasers, vehicle washes, disinfectants, sanitizers, laundry detergents and detergents for mechanical dish washers (Mueller et al. 2006; Glauser et al. 2007; Ahmed 2009). Other applications of NTA in Canada involve its function as a chelating agent in a variety of industrial processes, such as the treatment of boiler water and in pulp and paper processes to produce paper and paperboard products (Eichinger 2005; Gousetis and Opgenorth 2005; Bellows 2006; Glauser et al. 2007; 2009 email from Food Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced). Previously, NTA was proposed in the scientific literature as a therapeutic chelating agent for manganese poisoning and for the treatment of iron overloading (Kaur et al. 1980; Pollack and Ruocco 1981).

The total reported uses of NTA in Canada under section 71 for the year 2006 were in the range of 1000–10 000 kg (Environment Canada 2009a). Based on the available data in a technical report, all quantities of NTA (including its salts) imported into Canada in 2006 (i.e., 3.9 million kilograms) were consumed in the same year (Glauser et al. 2007).

Similar to the situation in Canada, the worldwide use of NTA is also concentrated in institutional and industrial cleaners. In addition, globally this substance is employed as a water softener for a variety of industrial applications, such as prevention of mineral scale formation in boiler water, leather tanning, textile treatment and cleaning, metal plating and cleaning, rubber production and purification of rare earth metals (Gousetis and Opgenorth 2005; NTP 2005; Bellows 2006; Glauser et al. 2007).

At one time, Na_3NTA was known for its use as a builder in laundry detergents, replacing phosphates when eutrophication became a worldwide concern (Lynn 2009). Starting in the 1990s, the use of Na_3NTA in laundry detergents declined in Canada (Suresh and Kishi 2006). Based on the available information, it is expected that the vast majority of household laundry products in the current Canadian market do not contain Na_3NTA (Modler et al. 2007). On the other hand, this substance has been identified in a few household cleaning products (SCJGSARA 2009; 2009 emails from Risk Management

Bureau, Health Canada, to Risk Assessment Bureau, Health Canada). Currently, major applications of Na_3NTA in Canada are similar to those of NTA: industrial and institutional cleaners and as a water softener in industrial processes. Na_3NTA is also used in pesticide formulations and personal care products in Canada and elsewhere (Gousetis and Opgenorth 2005; Glauser et al. 2007; PMRA 2007; CNS 2009).

Neither NTA nor Na_3NTA appears on Health Canada's Cosmetic Ingredient Hotlist, an administrative list of ingredients that are intended to be prohibited or restricted for use in cosmetics in Canada (Health Canada 2007). Na_3NTA was not identified in cosmetics via the section 71 survey, but it is currently listed in 11 personal care products on Health Canada's Cosmetic Notification System (CNS 2009).

In Canada, NTA is not used as a formulant or an active ingredient in any registered pest control products in Canada. However, Na_3NTA is used as a formulant in pest control products (PMRA 2007), but is not registered as an active ingredient under the Registered Products Database (PMRA 2009; 2009 emails from Pest Management Regulatory Agency, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced).

NTA is not listed in the Drug Products Database (DPD), the Natural Health Products Ingredients Database (NHPID) nor the Licensed Natural Health Products Database (LNHPD) as a medicinal ingredient or non-medicinal ingredient present in final pharmaceutical products, natural health products nor veterinary drugs manufactured in Canada (2008 emails from Natural Health Products Directorate and Veterinary Drugs Directorate, Health Canada, to Risk Management Bureau, Health Canada; 2009 emails from Therapeutic Products Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced). Na_3NTA is also not listed in the NHPID and the LNHPD, but it is listed as a non-medicinal ingredient in disinfectant products (2009 emails from Risk Management Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced).

NTA is a known impurity in ethylenediaminetetraacetic acid (EDTA) and its salts, collectively known as edetates (Dow Chemical Company 1987; Crosbie et al. 2003; Hart 2005). Edetates are chelating agents that may be used in household cleaning products, cosmetic formulations, foods, agricultural products, pharmaceutical products and industrial processes (Hart 2005). Edetates are also used to treat heavy metal poisoning and to reduce blood cholesterol (Lanigan and Yamarik 2002). According to the US Pharmacopeia (USP 2000a, b, c) and European Pharmacopoeia (EP 2001) and the Food Chemicals Codex (2009 emails from Food Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced), the maximum limit of NTA in edetates is 0.1% by weight.

Releases to the Environment

Consistent with the available technical literature (Glauser et al. 2007; Deligiannis and Piccione 2008), responses to the section 71 notice of CEPA 1999 indicated that NTA was

not manufactured in Canada in 2006. It is expected that the presence of NTA (and its salts) in the environment is predominantly a result of its release in municipal wastewaters through its use as a chelating agent or water softener in various applications (IJC 1977; Health Canada 1990).

According to the information gathered under CEPA 1999 through the section 71 notice with respect to NTA, companies reported no release of this substance to air or land in 2006, but 1000–10 000 kg of NTA was released to water in the same year. Responses to the section 71 notice also reported a transfer of 145–14 500 kg of NTA as hazardous or recycled waste to off-site waste management in 2006.

Information on the release and disposal of NTA in Canada can also be found in the National Pollutant Release Inventory (NPRI). Note that the figures reported by the NPRI on NTA may also include released and disposed quantities of NTA salts. The total on-site releases and total off-site disposals of NTA (and its salts) are tabulated in Table 3. Where information was provided, the NPRI indicates that on-site releases of NTA (and its salts) in Canada were solely to air, most likely through particulates, considering the physical and chemical properties of NTA and its salts. The total on-site release reported in 2006 was 177 kg, which is less than what was reported in responses to the section 71 notice. The total off-site disposal reported in 2006 was 5800 kg, which was sent to municipal sewage treatment plants for final disposal (NPRI 2006).

Table 3. NPRI release and disposal data for NTA (and its salts), 1994–2007[1,2]

Year	On-site releases (kg)				Total on-site releases (kg)	Total off-site disposals (kg)
	To air	To water	To land	To unspecified compartment		
1994	–	–	–	1 000	1 000	549
1995	25	–	–	601	626	2 000
1996	25	–	–	621	646	1 600
1997	2 600	–	–	300	2 900	2 900
1998	1 700	–	–	100	1 800	1 800
1999	1 900	–	–	100	2 000	3 300
2000	2 000	–	–	100	2 100	18 000
2001	1 500	–	–	0	1 500	14 000
2002	1 600	–	–	0	1 600	2 600
2003	1 300	–	–	0	1 300	7 300
2004	1 300	–	–	200	1 500	6 500
2005	–	–	–	299	299	14 000
2006	–	–	–	177	177	5 800
2007	–	–	–	536	536	5 400

[1] The NPRI reporting threshold is 10 000 kg of manufacturing, processing, or otherwise use activities.
[2] NPRI captured only a fraction of the total used quantity of NTA and salts reported elsewhere (Glauser et al. 2007).

Environmental Fate

NTA is present in water primarily in the form of metal complexes, rather than as the free acid (Health Canada 1990). The metal complexes formed depend on the composition (including metal content) of the water. For example, based on one model of NTA metal ion speciation, it was predicted that at a concentration of 25 µg/L in a river water, 50% of the NTA is complexed with copper ions, 34% with nickel ions, 9% with calcium ions and 5% with zinc ions (McFuff and Mord 1973).

Based on its physical and chemical properties (Table 2), the results of Level III fugacity modelling (Table 4) indicate that the neutral form of NTA is expected to reside predominantly in water or soil, depending on the compartment of release.

Table 4. Results of Level III fugacity modelling for the neutral (un-ionized) form of NTA (EQC 2003)

Substance released to:	Fraction of substance partitioning to each medium (%)			
	Air	Water	Soil	Sediment
Air (100%)	0.0	20.4	79.6	0.04
Water (100%)	0.0	99.8	0.0	0.2
Soil (100%)	0.0	16.1	83.8	0.03

Because the presence of NTA in the environment is predominantly from its release in municipal wastewater, the focus of the ecological assessment is on water and aquatic organisms.

Persistence and Bioaccumulation Potential

Environmental Persistence

Fischer et al. (1974) reported a 30-day biological oxygen demand (BOD) in water for NTA of 0–4% of the theoretical maximum oxygen demand. This test (which was the basis for decision regarding persistence for Categorization) suggests that the half-life of NTA in water is longer than 182 days (6 months), the criterion for persistence in water specified in the *Persistence and Bioaccumulation Regulations* (Canada 2000). On the other hand Health Canada (1990) reported that based on available empirical data, NTA biodegrades readily with a disappearance time in lakes and rivers of 11 days or less, depending on such factors as concentration of NTA and acclimatization of microorganisms. However, since this is a field study where test conditions could not be strictly controlled, it is possible that other loss processes than biodegradation were involved in the disappearance of NTA. The results of Fischer et al. (1974) are contrary to most of the results of other degradation studies, which indicate that NTA biodegrades very quickly under aerobic conditions. It is possible that results of Fischer et al. (1974)

were influenced by the formation of complexes with heavy metals which may inhibit NTA degradation (EURAR 2005).

A QSAR-based weight of evidence approach (Environment Canada 2007) was also applied using the degradation models shown in Table 5. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that NTA is expected to be released to this compartment, primarily biodegradation in water was examined. Table 5 summarizes the results of available QSAR models for degradation in various environmental media.

Table 5. Predicted data for degradation of NTA

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Air			
Atmospheric oxidation	AOPWIN 2008	$t_{½}$ = 1.47 h	<2
Ozone reaction	AOPWIN 2008	n/a[1]	n/a
Water			
Hydrolysis	HYDROWIN 2008	n/a[1]	n/a
Biodegradation (aerobic)	BIOWIN 2008 Submodel 3: Expert Survey (ultimate biodegradation)	3.62[2] "biodegrades very fast"	<182[3]
Biodegradation (aerobic)	BIOWIN 2008 Submodel 4: Expert Survey (primary biodegradation)	4.44[2] "biodegrades very fast"	<182[3]
Biodegradation (aerobic)	BIOWIN 2008 Submodel 5: MITI linear probability	0.75[4] "biodegrades very fast"	<182[3]
Biodegradation (aerobic)	BIOWIN 2008 Submodel 6: MITI non-linear probability	0.7[4] "biodegrades fast"	<182[3]
Biodegradation (aerobic)	CATABOL ©2004–2008 % BOD	% BOD = 0 "biodegrades slowly"	>182[3]

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade & Industry, Japan; n/a, not applicable; $t_{½}$, half-life.

[1] Model does not provide an estimate for this type of structure.

[2] Output is a numerical score.

[3] Expected half-lives for BIOWIN and CATABOL models are determined based on Environment Canada (2009b).

[4] Output is a probability score.

Based on the available modeling results and the information reported in Health Canada (1990), it is concluded that the half-life of NTA in water is much less than 182 days. Using an extrapolation ratio of 1:1:4 for a water: soil: sediment biodegradation half-life (Boethling et al. 1995), the half-life in soil is also <182 days and the half-life in sediments is <365 days. This indicates that NTA is not expected to be persistent in soil and sediment.

In air, a predicted atmospheric oxidation half-life of 1.47 hours (Table 5) demonstrates that this substance is likely to be rapidly oxidized. NTA is therefore considered to be not persistent in air.

In summary, the empirical data presented above (Fischer et al. 1974) suggest that the half-life of NTA in water is longer than 182 days (6 months). This result contradicts other reports of the disappearance of NTA from river and lake water in a matter of a few days and most of the model predictions presented in Table 5, which indicate that the substance would biodegrade quickly. Based on other empirical studies, the consistency of model predictions and considering the chemical structure of NTA, which contains easily biodegraded fragments, it is concluded that NTA does not meet the persistence criteria in water, soil or sediment (half-lives in soil and water ≥182 days and half-life in sediment ≥365 days), nor does it meet the criterion for air (half-life in air ≥2 days), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

Predicted log K_{ow} values for NTA suggest that this chemical has low potential to bioaccumulate in biota (see Table 2 above).

Limited data on the bioaccumulation of Na_3NTA summarized in EURAR (2005) indicate BCFs for several aquatic species including fish are typically less than 10 L/kg. Since few experimental bioaccumulation factor (BAF) or bioconcentration factor (BCF) data for NTA were available, a predictive approach was applied using available BAF and BCF models, as shown in Table 6. According to the *Persistence and Bioaccumulation Regulations* (Canada 2000), a substance is bioaccumulative if its BCF or BAF is ≥5000; however, measures of BAF are the preferred metric for assessing the bioaccumulation potential of substances. Kinetic mass balance modelling is in principle considered to provide the most reliable prediction method for determining the bioaccumulation potential, because it allows for metabolism correction as long as the log K_{ow} of the substance is within the log K_{ow} domain of the model.

Table 6. Fish BAF and BCF predictions for NTA

Test organism	Endpoint	Value (L/kg wet weight)	Reference
Fish	BAF	0.93	Arnot and Gobas 2003 (BAF middle trophic level)
Fish	BCF	0.93	Arnot and Gobas 2003 (BCF middle trophic level)
Fish	BCF	8.5	OASIS Forecast 2005
Fish	BCF	3.2	BCFBAF 2008

The predicted data presented in Table 6 are consistent with the empirical data for Na_3NTA, indicating that NTA does not have the potential to bioconcentrate in fish or to biomagnify in food webs. The Gobas middle trophic level BCF and BAF predictions include biotransformation rate estimates (k_M = 125 for 10 g fish). Health Canada (1990) however reported that NTA does not appear to be metabolized by mammals, in which

unchanged NTA is excreted in urine. The BCF of 3.2 L/kg, estimated by BCFBAF (2008) and presented in Table 6, does not include biotransformation.

Based on the available evidence, NTA does not meet the bioaccumulation criteria (BAF or BCF ≥5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

The approach taken in this ecological screening assessment was to examine various supporting information and develop conclusions based on a weight of evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from a conservative risk quotient calculation as well as information on persistence, bioaccumulation, toxicity, sources and fate of the substance.

As described previously, NTA is not persistent in water, soil or sediments. It is expected to have a low bioaccumulation potential.

A summary of experimental ecological effects data is presented in Table 7. These data indicate that NTA is not highly hazardous to aquatic organisms, with acute and chronic toxicity values >1 mg/L.

Table 7. Empirical data for aquatic toxicity

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Alga	Chronic (5–8 days)	LOEC	5–510	ECOTOX 2006
Daphnia	Acute (24 h)	EC_{50}	79–950	ECOTOX 2006
Fish (carp)	Acute (48 h)	LC_{50}	475	ECOTOX 2006
Amphibian (leopard frog)	Acute (120 h)	EC_{50}	60.4	ECOTOX 2006

Abbreviations: EC_{50} (median effective dose), the concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms; LC_{50} (median lethal concentration), the concentration of a substance that is estimated to be lethal to 50% of the test organisms; LOEC (lowest-observed-effect concentration), the lowest concentration in a toxicity test that caused a statistically significant effect in comparison with the controls.

The toxicity of the trisodium salt of NTA, CAS RN 5064-31-3 is quite similar to that of NTA. Algae (*Anabaena flosaquae, Selenastrum capricornutum* and *Anacystis aeruginosa*), with a 12-day toxicity of 5 mg/L (Sturm and Payne 1973) are the most sensitive aquatic organisms. For fish, 96-h LC_{50}s for trisodium NTA range from about 100 mg/L to >300 mg/L (Sturm and Payne 1973, Birge et al. 1979).

Since a significant proportion of NTA may be released to water, a generic scenario was used to estimate a conservative concentration of NTA resulting from an industrial discharge using Environment Canada's Industrial Generic Exposure Tool – Aquatic (IGETA). This yielded a predicted environmental concentration (PEC) of 0.05 mg/L.

Details regarding the inputs used to estimate this concentration and the output of the model are described in Environment Canada (2009c).

Older monitoring data are available for Canadian surface waters and municipal treatment plant (STP) effluents. Matheson (1977) presented monitoring data on NTA in receiving water from across Canada from 1971 - 1975. The highest reported concentration was 0.35 mg/L, in a sample from Hamilton harbour, Ontario. Woodiwiss et al. (1979) reported concentrations of NTA in streams in southern Ontario for the period 1971 – 1975. The highest concentration was 3.36 mg/L in a creek below a sewage outfall. Woodiwiss et al. (1979) reported concentrations up to 10.5 mg/L in effluents from 12 communities in southern Ontario. Matheson (1977) reported concentrations up to 2.8 mg/L in effluent from the Hamilton, Ontario, STP in 1973, before secondary treatment was installed at the facility. In these studies, the reported NTA concentrations would have resulted from usage of NTA itself and of its sodium salt. In addition they are not expected to be representative of current concentrations since the Canadian market use of NTA and its salts in 2006 (3.9 million kilograms; Glauser et al. 2007) is approximately seven times lower than that in 1977 (IJC 1977).

More recent and representive monitoring data were identified only for Canadian municipal STP effluents. Lee et al. (1996) reported concentrations of NTA up to 0.589 mg/L in final effluent from eight southern Ontario municipal STP effluents as well as selected paper mills in Ontario and Quebec. Assuming a dilution factor of 10, these data suggest that concentrations in Canadian surface waters resulting from the use of NTA and its salts may be as high as 0.059 mg/L.

A conservative predicted no-effect concentration (PNEC) was also derived from the lowest toxicity value. The chronic lowest-observed-effect concentration (LOEC) for three species of green algae was reported by Millington et al. (1988) to be 5 mg/L. This value was selected as the critical toxicity value and divided by an assessment factor of 10 to account for uncertainties related to interspecies and intraspecies variability in sensitivity and extrapolation from a laboratory LOEC to a no-effect value in the field. This yielded a PNEC of 0.5 mg/L.

The resulting conservative risk quotient (PEC/PNEC) of 0.1 (0.05/0.5) indicates that exposures are unlikely to be high enough to cause harm to aquatic organisms. Since the majority of releases of this substance are likely to water at industrial manufacturing sites and as the results of fugacity modelling indicate that most of the substance discharged to water will remain in that compartment, significant exposure of organisms at other types of locations or in media other than water is unlikely.

NTA is thus unlikely to be causing ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

Conservative assumptions were made in response to uncertainties encountered in the assessment. A key uncertainty relates to the lack of recent empirical data on surface water

concentrations in Canada, which was addressed by predicting a PNEC concentration in water using an industrial exposure model.

There are uncertainties associated with the use of QSAR models to estimate persistence and bioaccumulation. In addition, estimated values for some of the key physical and chemical properties, including vapour pressure and K_{ow}, were used as inputs for models.

There is also uncertainty about the critical toxicity study. The LOEC reported by Millington et al. (1988), 5 mg/L, was the lowest test concentration, so there was no no-observed-effect concentration (NOEC). Furthermore, the study did not report the degree of reduction in growth of the algae, just the fact that the reduction was statistically significant ($P = 0.95$) compared with controls. This makes it impossible to judge whether the PNEC represents a toxic threshold concentration.

Potential to Cause Harm to Human Health

Since the toxicological endpoints of NTA and its sodium salts are similar, and since the dissociation of NTA and its sodium salts eventually leads to a common moiety, nitrilotriacetate, it is deemed that nitrilotriacetate is a possible common moiety of toxicological interest (see Figure 1); thus toxicological studies using NTA and Na_3NTA were considered in this screening assessment. Data from studies with salts formed with various cations such as calcium, magnesium, aluminum, zinc and iron were not included. The European Union also similarly did not include these other NTA salts in their draft risk assessment report (EURAR 2008).

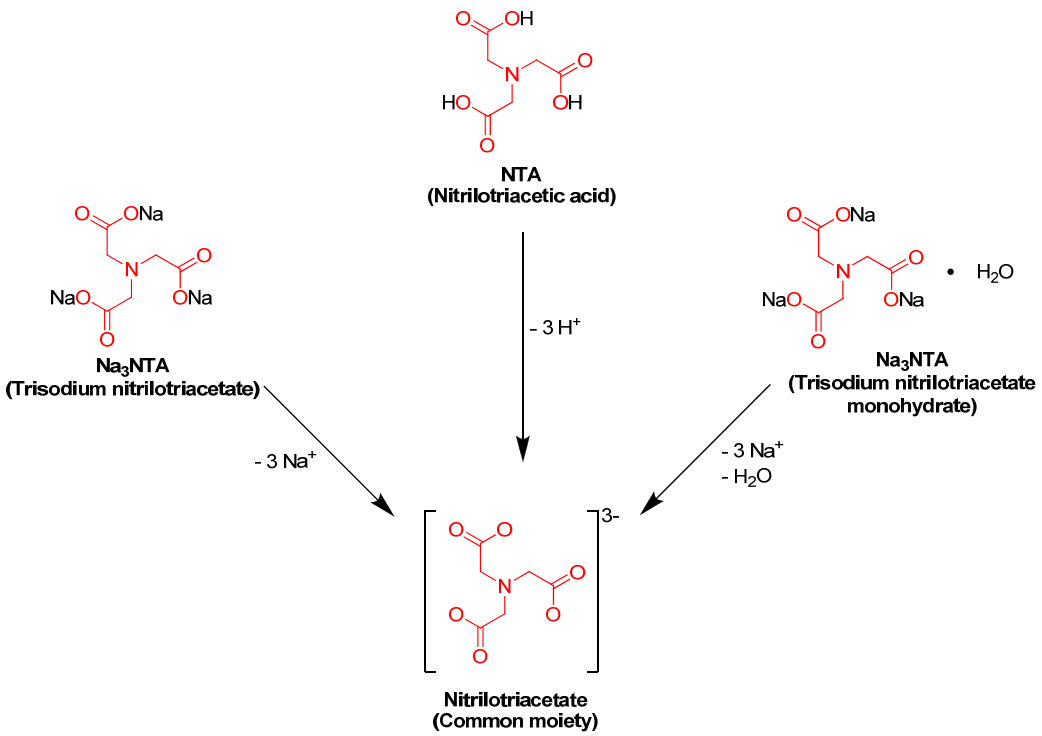


Figure 1. Dissociation of NTA and Na_3NTA into respective cations and nitrilotriacetate, a common moiety of toxicological interest

In consumer products, exposure estimations were derived for nitrilotriacetate of which NTA and Na_3NTA are the contributing forms. In environmental media, NTA and Na_3NTA cannot be distinguished. This is because NTA and Na_3NTA are converted into a common ester derivative and it is the ester derivative that is measured analytically.

While this assessment includes information on NTA and its sodium salts, the conclusion of this assessment pertains to NTA only; the information presented relating to Na_3NTA serves only as supporting material.

Exposure Assessment

Environmental Media and Food

As mentioned previously, NTA and Na_3NTA cannot be distinguished analytically in environmental measurements. For the sake of simplicity and since the analyte cannot be distinguished, the chemical species quantified during chemical analysis will be referred to as NTA in this section.

No data were identified regarding measured concentrations of NTA in air, soil or food. In contrast, there are a number of studies that have measured concentrations of NTA in Canadian municipally treated drinking water, surface water, groundwater and industrial water (Matheson 1977; Malaiyandi et al. 1979; Anderson et al. 1985).

A national monitoring program surveyed the NTA content in finished drinking water and groundwater in municipalities across Canada from 1972 to 1975 in order to investigate the environmental and public health effects of replacing phosphates in laundry detergents with Na_3NTA (Matheson 1977). In the early 1970s, Na_3NTA was contained in Canadian household laundry formulations at an average concentration of 6% by weight (Anderson et al. 1985). In treated municipal drinking water, NTA was not detected in most cases (limit of detection 10 µg/L); the highest concentration measured, 80 µg/L, was found in a sample from Granby, Quebec, in 1975. Similarly, NTA was not detected in most samples from municipal groundwater supplies.

A separate Canadian survey was conducted by Malaiyandi and co-workers from 1976 to 1977 during the winter, a season when higher levels of NTA in water were expected due to reduced biodegradation (Malaiyandi et al. 1979). During the time of this survey, the average level of Na_3NTA in laundry detergents in Canada was 15% by weight (Anderson et al. 1985). The content of NTA in drinking and raw water was determined in 70 municipalities across the country. The national mean concentration of NTA in drinking water was 2.82 µg/L; the measurements ranged from below the limit of detection (0.2 µg/L) to 20.4 µg/L. The average concentration of NTA in water supplies derived from private wells was 3.88 µg/L (range <0.2–33.5 µg/L).

For 10 Canadian cities, Malaiyandi and co-workers compared their 1976–1977 measurements with the data previously collected in 1972–1975 (Matheson 1977) and

showed that the more recent NTA concentrations were generally less than or equal to earlier measurements. This suggested that NTA was not accumulating in Canadian drinking water supplies, although the Na_3NTA content in domestic laundry formulations had increased. This was reaffirmed in a separate Canadian study in 1983 that measured concentrations of NTA in private wells in Ontario and British Columbia (Procter & Gamble Company 1983a). Seventy-five percent of the samples had NTA concentrations below the limit of detection (1 µg/L); one sample in Ontario was measured at 2.7 µg/L, and the remaining samples, located in British Columbia, contained an average concentration of 3.6 µg/L.

The maximum concentration of NTA in treated drinking water in the 1976–1977 Canadian survey, 20.4 µg/L (Malaiyandi et al. 1979), was used to derive exposure estimates. For comparison, the maximum acceptable concentration of NTA in drinking water defined in the Guidelines for Canadian Drinking Water Quality is 400 µg/L (Health Canada 2008).

Concentrations of NTA in treated drinking water and groundwater were also measured in the United States in the early 1970s (not detected in most samples; 4.6% of the samples contained concentrations between 25 and 125 µg/L) and early 1980s (range <1–7 µg/L) and in Europe during the 1990s (range <1–3 µg/L). Similar to the results from the Canadian surveys, NTA was not detected in the majority of the American studies, while higher concentrations were found in shallow wells (Procter & Gamble Company 1972, 1981, 1982, 1983b, c; Stabel 1998; Schmidt et al. 2004).

NTA has also been measured in untreated US and European surface waters (Voulgaropoulos and Tzivanakis 1992; Pietsch et al. 1995; Kiessling and Kaluza 1997; Ding et al. 1999; Schmidt et al. 2004) and in industrial waters in Canada (range <0.5–>10 000 µg/L), the United States (range 30–2400 µg/L) and Europe (typical effluent concentration range 100–2000 µg/L) (Matheson 1977; Woodiwiss et al. 1979; Anderson et al. 1985; Voulgaropoulos and Tzivanakis 1992; Garric et al. 1996; Lee et al. 1996; Knepper 2003; EURAR 2005).

Industrially, NTA is used as a water softener in pulp and paper processing to produce paper and paperboard products, such as those used for food packaging (Eichinger 2005). It is estimated that the levels of NTA in these finished packaging products are minimal; thus, any exposure through migration into foods in this case would be negligible (2009 email from Food Directorate, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced).

NTA is also used as a descaling agent in the treatment of boiler water to prevent mineral scale formation (Gousetis and Opgenorth 2005). This application can be found in the food industry where steam is generated and potentially contacts food during processing (AMT 1995; Bellows 2006). Since NTA has a measured melting point of 242ºC and an estimated boiling point of 429ºC, it is expected that there would be minimal contamination of foods with NTA during food processing involving steam (2009 email

from Food Directorate, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced).

NTA may be present as an impurity in both disodium and calcium disodium salts of food-grade EDTA, which are permitted for use as sequestering agents in a number of foods as per the *Food and Drug Regulations*. The regulations require that food additives meet the purity specifications outlined in the Food Chemicals Codex. Accordingly, food-grade EDTA must not contain more than 0.1% NTA by weight. Given the maximum limit for NTA in food-grade EDTA and the maximum levels of use for EDTA in foods specified by the *Food and Drug Regulations*, exposure to NTA from food, if any, is expected to be negligible (2009 email from Food Directorate, Health Canada, to Risk Management Bureau, Health Canada; unreferenced).

Upper-bounding estimates of daily exposure to NTA for various age groups of the general population are shown in Appendix 1. These estimates were derived from the maximum concentration of NTA in treated drinking water from the 1976–1977 Canadian survey, 20.4 µg/L (Malaiyandi et al. 1979), and range from 0.41 µg/kg body weight (kg-bw) per day (12–19 years of age) to 2.18 µg/kg-bw per day (formula-fed infants 0–6 months of age). The estimates presented in Appendix 1 are expected to be conservative and overestimations of human exposure to NTA. This is because Na_3NTA was used extensively as a replacement for phosphate builders during the time of the Canadian surveys in the 1970s, but its use in Canadian laundry detergents declined sharply in the mid-1990s (Suresh and Kishi 2006; Glauser et al. 2007; Zoller 2009). The Canadian market use of NTA and its salts in 2006 (3.9 million kilograms) is approximately seven times lower than that reported in 1977 (>27 million kilograms) (IJC 1977). This may relate to the observed general trend of decreasing NTA levels in various water sources.

Confidence is high that dietary exposure is negligible for all age groups and that the resulting upper-bounding exposure estimates are protective of the general population of Canada. This is because exposure estimates were derived using the maximum concentration of NTA (20.4 µg/L) in drinking water from the 1976–1977 Canadian survey (Malaiyandi et al. 1979). Furthermore, current drinking water levels are anticipated to be lower based on the significantly reduced usage of NTA since the 1970s. Confidence in the estimated NTA exposure through intake of drinking water is considered to be moderate, as the Canadian monitoring data for NTA in municipally treated drinking water are very extensive but outdated and representative of a period of high NTA usage. Although no data were available on the concentration of NTA in other environmental media, it is expected that drinking water contributes the most to human exposure due to the physical and chemical properties of NTA and its widespread use as a water softener.

Consumer Products

Based on the available information, Na_3NTA is used more commonly than NTA in consumer products.

Na_3NTA has been found in the personal care products listed in Table 8. These products are considered to be used frequently (CNS 2009). Maximum concentrations reported in these products were used to derive exposure estimates from consumer use. Dermal and inhalation exposures were estimated using ConsExpo 4.1 (ConsExpo 2006), and details are included in Appendix 2.

In a metabolism study of disodium NTA given orally to a monkey test animal, 14% of the administered dose was recovered from its urine (Michael and Wakim 1971). In a separate human metabolic study, the oral absorption of nitrilotriacetate was reported to be 12% (based on concentration in urine), and total recovery from urine, feces and expired carbon dioxide was 89% in male volunteers subjected to single oral doses of 10 mg $^{14}C[Na_3NTA]$ (Budny and Arnold 1973). Skin penetration of Na_3NTA was investigated *in vitro* by the European Union using radiolabelled Na_3NTA (EURAR 2008). In their draft risk assessment report (EURAR 2008), absorption of Na_3NTA from dilute solutions ranged from 0.042% to 0.472%; thus, the European Union used a conservative absorption of 1% for risk characterization of dilute solutions of Na_3NTA. For products with higher Na_3NTA concentrations, the European Union used a higher absorption of 10% (default based on physicochemical properties). Based on the weight of evidence from the apparent low oral absorption in primates and the low dermal absorption noted *in vitro*, a conservative dermal absorption of 10% was selected for this screening assessment.

Table 8. Estimated exposures[1] to Na_3NTA (maximum concentration 0.1% by weight) from personal care products that are used frequently (CNS 2009)

Consumer product	Use frequency (times per year)	Dermal[2] (mg/kg-bw per day)
Face makeup pressed powder (women only)	365	0.00425
Fragrance[3]	1095	0.00145
Hair shampoo	260	0.0201
Skin cleanser for body	329	0.000619
Skin cleanser for face	730	5.65×10^{-5}
Total[4]		0.0265 Na_3NTA or 0.0197 NTA

[1] Body weight assumed to be 70.9 kg (Health Canada 1998).
[2] Assuming 10% dermal absorption (EURAR 2008).
[3] The inhalation exposure through usage of fragrance was estimated to be minimal (5.24×10^{-7} mg/kg-bw per day).
[4] Calculated for women using face makeup pressed powder as the upper-bounding scenario.

The vapour pressure of NTA and its salts is very low; therefore, inhalation exposure to NTA and its salts evaporated from solutions is estimated to be negligible (see Appendix 2). This is consistent with other risk assessments of NTA salts, which assumed negligible inhalation exposure (CanTox Inc. 1996; EURAR 2008). An exception occurs when formation of aerosols is possible, such as in the use of spray products, which was considered in this assessment

The maximum exposure estimated from the use of a personal care product listed in Table 8 is 0.0201 mg/kg-bw per day, which is through dermal absorption from the use of hair

shampoo. The total exposure to Na_3NTA is 0.0265 mg/kg-bw per day; converting this total into NTA equivalents, for the purpose of subsequent margin of exposure calculations, yields 0.0197 mg/kg-bw per day.

Na_3NTA was also identified in a multi-purpose household cleaning product. Exposure to Na_3NTA in this product was estimated to be negligible (see Appendix 2).

In addition to the frequently used personal care products listed in Table 8, Na_3NTA has been found in other consumer products listed in Table 9, which are considered to be used occasionally. Exposure estimates were derived from maximum concentrations reported in these products (CNS 2009).

Table 9. Estimated exposures[1] to Na_3NTA from consumer products that are used occasionally

Consumer product	Na_3NTA maximum concentration (% by weight)	Use frequency (/year)	Mean event concentration (mg/m^3)	Dermal exposure[4] (mg/kg-bw)
Hair dye, semi-permanent[2]	0.3	13	NA	0.000685
Foot lotion for pedicure (women only)[2]	0.1	156	NA	0.00656
Nail polish top coat for manicure (women only)[2]	0.1	156	NA	0.000121
Bathroom cleaner[3]	1	52	0.000229 (s)	0.000973 (s) 0.00423 (c)
Total			0.000229	0.0126

Abbreviation: NA, not applicable

[1] Body weight assumed to be 70.9 kg (Health Canada 1998).

[2] CNS (2009).

[3] SCJGSARA (2009). Exposures estimated from spraying (s) and cleaning (c).

[4] Assuming 10% dermal absorption (EURAR 2008).

The highest exposure from a single consumer product listed in Table 9 is 0.00656 mg/kg-bw through use of foot lotion for pedicures. The confidence in this estimate is low because there are no data on the use pattern of foot lotion for pedicures.

Potential inhalation and dermal exposures from usage of insecticide or miticide pest control products containing Na_3NTA are estimated to be minimal. Potential dermal and oral exposures resulting from application of a swimming pool algicide containing Na_3NTA are estimated to be minimal (refer to Appendix 2).

All consumer products mentioned thus far in this section contain Na_3NTA. As previously mentioned in the "Uses" section, NTA is used in photographic developer solutions. These products are considered to be of occupational and possibly hobbyist usage and therefore are not expected to contribute to the general population exposure. It was also mentioned that NTA is a common impurity in edetates. Based on the available information, the introduction of NTA in consumer products is usually a consequence of such

contamination. It is expected that only trace residual levels of NTA would be found in edetate-containing consumer products, such as fertilizers, the only known type of consumer product that lists NTA in the product composition. Exposures to NTA from trace impurities in these products were estimated to be minimal (see Appendix 2).

Thus, confidence in the numerical results of the exposure estimations arising from consumer products is moderate to low in the absence of experimental data, but confidence is high that these products result in minimal exposure to NTA and its salts.

Health Effects Assessment

A summary of the available health effects information for NTA and its sodium salts is presented in Appendix 3.

On the basis of investigations in experimental animals, NTA and its sodium salts have been classified by the International Agency for Research on Cancer (IARC 1990, 1999) as a Group 2B carcinogen ("possibly carcinogenic to humans"). The European Commission has classified Na_3NTA (CAS RN 5064-31-3) as a Category 3 carcinogen with the risk phrase R40 ("limited evidence of a carcinogenic effect") (European Commission 2007; EURAR 2008). The US National Toxicology Program considers NTA as "reasonably anticipated to be a human carcinogen" based on sufficient evidence of carcinogenicity in experimental animals (NTP 2005). In the Guidelines for Canadian Drinking Water Quality, NTA was classified in Group IIIB ("possibly carcinogenic to humans") "because NTA induces [renal] tumours only at doses higher than those that are nephrotoxic" (Health Canada 1990). The carcinogenicity of NTA and its sodium salts was demonstrated for the oral route; no long-term toxicity data were identified for the inhalation or dermal route (EURAR 2008).

Two bioassays for the carcinogenicity of Na_3NTA and one for NTA were conducted in F344 rats (NCI 1977). In one bioassay, a significant increase in the incidences of primary neoplasms of the urinary tract was observed in both sexes of rats at 20 000 ppm when exposed to Na_3NTA in the diet for 24 months. A marginal increase of hyperplastic and dysplastic lesions was seen at 200 ppm Na_3NTA (equivalent to 10 mg/kg-bw per day), and the increase was marked at 2000 ppm Na_3NTA (equivalent to 100 mg/kg-bw per day) and above.

In the two other bioassays, F344 rats were orally exposed to 0, 7500 or 15 000 ppm of Na_3NTA or NTA for 18 months followed by a 6-month recovery period. A significant increase in the incidences of primary neoplasms of the urinary tract in the NTA-treated groups and an increase in a similar spectrum of tumours (not statistically significant) in the Na_3NTA-treated groups in both sexes of animals were reported. Hepatocellular adenomas and adrenal phaeochromocytomas in high-dose NTA-treated females were also observed (NCI 1977). A significant increase in renal adenomas and adenocarcinomas was also observed in male Sprague-Dawley rats exposed to 0.1% Na_3NTA (equivalent to 100 mg/kg-bw per day) in their drinking water for 704 days (Goyer et al. 1981).

In mice, a significant increase in the incidence of kidney tumours, mainly tubular cell adenocarcinomas, was observed in high-dose females and both low- and high-dose males exposed to NTA at 0, 7500 or 15 000 ppm administered in feed for 18 months, followed by a 3-month recovery period. When mice were exposed to Na_3NTA at 0, 2500 or 5000 ppm in feed over the same dosing period, no urinary tract tumours were observed, but hematopoietic tumours were reported in treated males (NCI 1977).

For the urinary tract tumours, NCI (1977) suggested that they "may be due to a local effect which can be brought about only by high concentration [of NTA or Na_3NTA]." NCI (1977) did not draw a conclusion about the significance of the occurrence of tumours other than those of the urinary tract.

Tumour promotion effects of Na_3NTA and NTA were also reported in several two-stage initiation–promotion studies. Accelerated development of urinary system tumours was seen in mice and rats pretreated by the renal carcinogens, nitrosamines, and then treated orally with NTA or Na_3NTA for 28–30 weeks. Treatment-associated induction of hyperplasias of the urinary system for shorter time periods, compared with the long-term studies mentioned above (NTA or Na_3NTA administered alone), was also reported in these studies (Hiasa et al. 1984, 1985a, b; Kitahori et al. 1985, 1988; Matsuki et al. 1992).

In vitro, NTA and Na_3NTA were negative with respect to the induction of gene mutations in bacteria and fungi, whereas only NTA was negative in the SOS chromotest (see Appendix 3 for details and references). Na_3NTA did not induce gene mutation or cell transformation in mammalian cells (Celotti et al. 1987; Mitchell et al. 1988; Myhr and Caspary 1988), but positive results were observed with NTA (Nesslany et al. 2008). Mixed results for chromosome aberrations and micronucleus induction were observed in human lymphocytes exposed to NTA or Na_3NTA (Montaldi et al. 1987, 1988; Nesslany et al. 2008). Micronucleus induction was positive in mouse cell lines and in primary kidney cells and urothelium of humans and rats when exposed to NTA (Robbiano et al. 1999, 2002; Nesslany et al. 2008). NTA and Na_3NTA did not induce sister chromatid exchange or unscheduled DNA synthesis in mammalian cell lines (Brat and Williams 1984; Montaldi et al. 1985).

In *in vivo* studies, NTA and Na_3NTA did not induce mutations in *Drosophila melanogaster* germ cells but (weakly) induced meiotic aneuploidy and somatic mutation. The substances did not induce dominant lethal mutations, but they induced aneuploidy in mice spermatocytes after intraperitoneal injection. However, they did not induce aneuploidy, micronucleus induction or sister chromatid exchange in mouse bone marrow cells *in vivo*. Some studies found that NTA was able to induce DNA damage in rat kidney or urinary bladder *in vivo*, but non-standard test protocols were used, and the dose–response relationship was not clear (see Appendix 3 for details and references).

IARC (1990, 1999) concluded that "nitrilotriacetic acid and its sodium salts were not genotoxic in experimental systems *in vivo*, except that the acid induced aneuploidy in mouse germ cells. Neither the acid nor its salts were genotoxic in mammalian cells *in vitro* and they were not mutagenic to bacteria." Based on results observed in mammalian

somatic cells *in vitro* and *in vivo*, the European Union, in its draft risk assessment report, claimed that "there is no plausible evidence for *in vivo* mutagenicity of NTA and its sodium salts" (EURAR 2008).

The development and analysis of modes of action for toxicological effects due to NTA and its sodium salts are outside the scope of this screening assessment. However, a presumed mode of action for the induction of urinary tract tumours by NTA and its various sodium salts was proposed by the European Union in its draft risk assessment report (EURAR 2008). It was hypothesized that the carcinogenic potential of NTA and Na_3NTA is related to sustained stimulation of cell proliferation following cytotoxicity induced by the substances; this is based on no evidence of a direct genotoxic action due to NTA and an increased incidence of cytotoxicity/hyperplasia and tumours observed in identical urinary system target sites. Although there was no sufficient evidence for the contribution of metal cations and the formation of NTA complexes to the observed carcinogenicity, their involvement in disregulation of cell growth and manifestation of neoplastic cell growth may be suspected (EURAR 2008).

Limited data relating to reproductive performance were reported from a combined reproduction and teratology study. Results indicated that dietary administration of Na_3NTA to rats did not adversely affect reproductive performance and capability or induce embryonic or fetal effects through two successive generations. Similar results were observed in rabbits and mice (Nolen et al. 1971, 1972a, b; Tjälve 1972).

Hydropic degeneration of the kidney tubule and minor tubule dilatation started after 6 months in a 2-year study in rats (exposed to 0%, 0.03%, 0.15% or 0.5% Na_3NTA in their diet). At 24 months, moderate to severe chronic interstitial nephritis and nephrosis were observed, with a dose-related increase in incidence and severity at 0.15% and 0.5% Na_3NTA. An increased concentration of zinc in the urine was also noted at all doses except the low dose. An oral lowest-observed-adverse-effect level (LOAEL) for Na_3NTA of 97 mg/kg-bw per day (0.15% Na_3NTA) was derived from this study (Nixon et al. 1972). Similar oral LOAELs for Na_3NTA (approximately 100 mg/kg-bw per day) were derived from a 2-year and a 10-week drinking water study in Sprague-Dawley rats, as well as from a 2-year diet study in F344 rats (Mahaffey and Goyer 1972; NCI 1977; Goyer et al. 1981).

The studies mentioned above show that the organ system mainly affected by repeated oral treatment with NTA or Na_3NTA is the urinary system: lesions were observed in the kidneys, ureters and urinary bladders in rodents within weeks of dosing and increased in severity with duration of dosing (Nixon 1971; Mahaffey and Goyer 1972; Nixon et al. 1972; Alden et al. 1981; Merski 1982; Myers et al. 1982; Kanerva et al. 1984; BASF 1997a).

In a 4-week and a 3-month dermal rabbit study, there were no local irritations or systemic effects observed at a Na_3NTA dose of 50 mg/kg-bw per day (2.5% Na_3NTA, the only dose tested) (Nixon 1971).

Very limited information on NTA inhalation exposure was contained in correspondence from a submitter to the US Environmental Protection Agency (EPA). When exposed to NTA at 343 mg/m^3 for 4 weeks, treated monkeys exhibited diarrhea. However, no respiratory irritation or general discomfort was observed (US EPA 1980).

Limited information regarding the toxicity of Na_3NTA in humans was identified. No evidence of sensitization was noted in any of 66 human volunteers subjected to a closed patch test with a 1% aqueous solution of a liquid detergent containing 20% Na_3NTA (Nixon 1971).

The absorption, distribution and excretion of NTA and its sodium salts following oral administration were investigated in rats, mice, rabbits, dogs, monkeys and humans. Absorption from the gastrointestinal tract probably varies in different species: for example, absorption appears to be greater in rats and dogs than in rabbits or primates. Approximately 12% of the administered dose was excreted via urine in humans, 14% in monkeys, 23% in rabbits, 70% in rats, 80% in dogs and 96% in mice. NTA does not appear to be metabolized by mammals, based on the studies in mice, rats, dogs and humans, in which NTA itself is excreted in the urine. Absorbed parent was excreted rapidly via urine. Less than 1% of the administered dose was excreted in the expired air. Subsequent to absorption, the highest concentration of NTA was found in the kidneys, urinary bladders and skeletal tissues (Michael and Wakim 1971; Budny 1972; Tjälve 1972; Budny and Arnold 1973; Chu et al. 1978; Anderson et al. 1985; WHO 1996).

The confidence in the toxicity database for NTA and its sodium salts is considered to be moderate to high, as acute, short-term, subchronic and chronic toxicity, carcinogenicity, genotoxicity, and reproductive and developmental toxicity oral studies are available, although inhalation and dermal exposure studies (for carcinogenicity and reproductive and developmental toxicity) were limited.

Characterization of Risk to Human Health

Based principally on the weight of evidence–based assessments of international and other national agencies, a critical effect for the characterization of risk to human health for NTA is carcinogenicity. Incidences of urinary system tumours were increased in a dose-related manner in the standard carcinogenicity studies in both sexes of rats and mice. Based on the weight of evidence of the available genotoxicity data, NTA is not considered to be genotoxic. Although the mode of induction of tumours has not been fully elucidated, the tumours observed in experimental animals are unlikely to have resulted from direct interaction with genetic material (EURAR 2008). Therefore, a threshold approach is used to assess risk to human health.

Similar to the neoplastic lesions, NTA and Na_3NTA induced non-neoplastic lesions mainly in the kidneys, ureters and urinary bladders of experimental animals. Adverse pre-neoplastic lesions were noted to occur mainly at dose levels that induced tumour formation. A progression from pre-neoplastic lesions (degeneration, vacuolisation, hyperplasia and dysplasia of epithelia) to tumour development in the urinary system was

observed in NTA- and Na_3NTA-treated rats. Therefore, margins of exposure for human health characterization are derived from the lowest exposure levels associated with induction of these effects and the estimates of population exposure to NTA and Na_3NTA.

The lowest oral LOAEL for Na_3NTA of 100 mg/kg-bw per day (equivalent to 70 mg/kg-bw per day as NTA on a molar basis) from short-term and chronic studies was identified in the toxicity data set, based on hyperplasia of tubular and transitional cells in kidneys, ureters and urinary bladders of treated rats (Mahaffey and Goyer 1972; NCI 1977; Goyer et al. 1981). Hydropic degeneration of kidney tubule cells, minor tubule dilatation and increased concentration of zinc in the urine were also described in a chronic oral study at a similar dose level (equivalent to 97 mg/kg-bw per day as Na_3NTA or 68 mg/kg-bw per day as NTA) (Nixon et al. 1972). Renal adenomas and adenocarcinomas were also reported at a Na_3NTA dose of 100 mg/kg-bw per day in rats exposed to Na_3NTA in drinking water for 2 years (Goyer et al. 1981).

A principal route of exposure to NTA is expected to be the oral route, because the most relevant source of human exposure appears to be the consumption of drinking water, due to the physical and chemical properties of NTA. Comparison of the critical effect level for repeated-dose toxicity via the oral route (100 mg/kg-bw per day as Na_3NTA, equivalent to 70 mg/kg-bw per day as NTA) with the upper-bounding estimate of general population exposure via the oral route (2.18 µg/kg-bw per day as NTA) results in a margin of exposure of approximately 32 000.

Dermal exposure from personal care products containing Na_3NTA was also estimated (refer to "Exposure Assessment" section). Since no chronic dermal toxicity studies were identified, the critical effects noted in the chronic oral dosing studies were adjusted to compare with conservative dermal exposure estimates from the use of personal care products for derivation of the margin of exposure. The critical effect concentration (100 mg/kg-bw per day as Na_3NTA), when corrected for 10% dermal absorption (100 mg/kg-bw per day/10% = 1000 mg/kg-bw per day), produced an equivalent dermal LOAEL of 1000 mg/kg-bw per day as Na_3NTA (equivalent to 700 mg/kg-bw per day as NTA). Comparison of this value with a conservatively predicted chronic dermal exposure via frequently used personal care products (0.0197 mg/kg-bw per day as NTA) results in a margin of exposure of approximately 35 500.

Even if the very conservative LOEL of 10 mg/kg-bw per day as Na_3NTA (equivalent to 7 mg/kg-bw per day as NTA), based on the marginal increase of hyperplasia and dysplasia of urinary tract epithelial cells in experimental animals, is used for this purpose, the potential margins of exposure for both oral and dermal exposure are expected to be large (3200 and 3550, respectively).

Although dermal studies with shorter-term exposures were available, the highest doses tested resulted in NOAELs. Comparison of the NOAEL for subchronic exposure (50 mg/kg-bw per day as Na_3NTA, equivalent to 35 mg/kg-bw per day as NTA) with the conservatively predicted chronic dermal exposure (0.0197 mg/kg-bw per day as NTA) results in a margin of exposure of approximately 1770. If the shorter-term study was

extrapolated to a longer-term exposure, given the shallow dose–response in progression of toxicity with time (reduced by half from the short-term feeding study low-effect level), a hypothetical chronic NOAEL would be 25 mg/kg-bw per day as Na_3NTA (equivalent to 17.5 mg/kg-bw per day as NTA). Using this NOAEL (17.5 mg/kg-bw per day) results in a margin of exposure of approximately 900, which still would be considered adequately protective of human health.

Uncertainties in Evaluation of Risk to Human Health

The scope of this screening assessment does not include a full analysis of the mode of action of NTA, nor does it take into account possible differences in sensitivity between humans and experimental species. Although there is no plausible evidence for the *in vivo* mutagenicity of NTA and its sodium salts, some mixed results are also obtained in the genotoxicity data set. In addition, only limited information is available concerning the potential toxicity of NTA following inhalation and dermal exposures and for reproductive and developmental toxicity.

There is some uncertainty in the estimation of exposure to NTA from environmental media and food. No data were identified regarding measured concentrations of NTA in air, soil or food. However, exposure from these sources is expected to be negligible. Therefore, exposure estimates were derived solely from Canadian monitoring data in municipally treated drinking water from the 1970s. Considering that the data originate from extensive Canadian monitoring studies and that usage has decreased significantly since the 1970s, estimates of exposure to NTA are expected to be conservative.

There is uncertainty due to the limited information on the presence or concentrations of NTA and Na_3NTA in products available in Canada. Estimates of exposure from the use of consumer products containing NTA and Na_3NTA were based on conservative assumptions.

Conclusion

Based on the information presented in this final screening assessment, it is concluded that NTA is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the available information, it is concluded that NTA is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health. It is therefore concluded that NTA does not meet any of the criteria in section 64 of CEPA 1999.

This substance will be considered for inclusion in the *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

References

ACD/Labs. 2008. Software version 8.14 for Solaris. Toronto (ON): Advanced Chemistry Development.

ACD/pK_aDB [Prediction Module]. 2005. Version 9.04. Toronto (ON): Advanced Chemistry Development. Available from: http://www.acdlabs.com/products/phys_chem_lab/pka/. [restricted access]

Ahmed FU. 2009. Industrial and institutional cleaners. In: Zoller U, editor. Handbook of detergents. Part E: Applications. Surfactant Science Series, Vol. 141. Boca Raton (FL): Taylor & Francis. p. 201–286.

Alden CL, Kanerva RL, Anderson RL, Adkins AG. 1981. Short-term effects of dietary nitrilotriacetic acid in the male Charles River rat kidney. Vet Pathol 18: 549–559.

[AMT] Adalia Miljö-och toxikologkunsult. 1995. Nitrilotriacetic acid; trisodium nitrilotriacetate; trisodium nitrilotriacetate monohydrate. Täby (SE): Submission to Nordic Steering Group for Assessment of Health Effects of Chemicals.

Anderson C, Danylchuk KD. 1980. The effect of chronic administration of trisodium nitrilotriacetate (Na_3NTA) on the Haversian remodelling system in dogs. J Environ Pathol Toxicol 3: 413–420.

Anderson RL, Kanerva RL. 1978. Hypercalcinuria and crystalluria during ingestion of dietary nitrilotriacetate. Food Cosmet Toxicol 16: 569–574.

Anderson RL, Kanerva RL. 1979. Comparisons of response of Fischer-344 and Charles River rats to 1.5% nitrilotriacetic acid and 2% trisodium nitrilotriacetate, monohydrate. Food Cosmet Toxicol 17: 137–140.

Anderson RL, Bishop WE, Campbell RL. 1985. A review of the environmental and mammalian toxicology of nitrilotriacetic acid. CRC Crit Rev Toxicol 15(1): 1–102.

[AOPWIN] Atmospheric Oxidation Program for Windows [Estimation Model]. 2008. Version 1.92. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

Arnot JA, Gobas FAPC. 2003. A generic QSAR for assessing the bioaccumulation potential of organic chemicals in aquatic food webs. QSAR Comb Sci [Internet] 22(3): 337–345. Available from: http://www3.interscience.wiley.com/journal/104557877/home [restricted access]

Bahnemann R, Leibold E, Kittel B, Mellert W, Jäckh R. 1998. Different patterns of kidney toxicity after subacute administration of Na-nitrilotriacetic acid and Fe-nitrilotriacetic acid to Wistar rats. Toxicol Sci 46: 166–175.

BASF. 1985. Report on the study of acute oral toxicity (Test substance TRILON A 92). Unpublished report (84/238), dated March 18, 1985. Ludwigshafen (DE): BASF, Department of Toxicology. [cited in EURAR 2008].

BASF. 1997a. Screening tests for kidney toxicity with Trilon AS in Wistar rats. Project No. 25S0109/94021. Ludwigshafen (DE): BASF. [cited in EURAR 2008].

BASF. 1997b. Trilon A 92 - Buehler test in guinea pigs. Unpublished report (32H0061/952192), dated December 11, 1997. Ludwigshafen (DE): BASF, Department of Toxicology. [cited in EURAR 2008].

BASF. 2004. Cytogenetic study *in vivo* with Trilon A 92 R. Project No. 26M0192/024031. Ludwigshafen (DE): BASF. [cited in EURAR 2008].

[BCFBAF] BioConcentration Factor Program for Windows [Estimation Model]. 2008. Version 3.00. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

[BCR] BC Research Inc., Occupational and Environmental Risk Management Group. 2002. Material safety data sheet: Gen-X Micro [Internet]. Langley (BC): Grotek. [cited 2009 Aug 31]. Available from: http://www.grotek.net/products/msds.asp

Becking GC, Yagminas AP. 1978. Relative mammalian toxicity of fulvic acid and trisodium nitritriacetic acid (NTA). Can Fed Biol Soc Proc 21: 159.

Bellows J. 2006. Steam [Internet]. In: Kirk-Othmer encyclopedia of chemical technology, online version. Available from: http://mrw.interscience.wiley.com/emrw/9780471238966/kirk/article/steabell.a01/current/pdf [restricted access]

[BIOWIN] Biodegradation Probability Program for Windows [Estimation Model]. 2008. Version 4.10. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

Birge WJ, Black JA, Bruser DM. 1979. Toxicity of organic chemicals to embryo-larval stages of fish. EPA-560/11-79-007. U.S. Environmental Protection Agency, Washington DC. (OECDG Data File).

Boethling RS, Howard PH, Beauman JA, Larosche ME. 1995. Factors for intermedia extrapolations in biodegradability assessment. Chemosphere 30(4): 741–752.

Brat SV, Williams GM. 1984. Nitrilotriacetic acid does not induce sister-chromatid exchanges in hamster or human cells. Food Chem Toxicol 22: 211–215.

Budny JA. 1972. Metabolism and blood pressure effects of disodium nitrilotriacetate (Na_2NTA) in dogs. Toxicol Appl Pharmacol 22: 655–660.

Budny JA, Arnold JD. 1973. Nitrilotriacetate (NTA): human metabolism and its importance in the total safety evaluation program. Toxicol Appl Pharmacol 25: 48–53.

Budny JA, Niewenhuis RJ, Buehler EV, Goldenthal EI. 1973. Subacute oral toxicity of trisodium nitrilotriacetate (Na_3NTA) in dogs. Toxicol Appl Pharmacol 26: 148–153.

Canada. 1999. *Canadian Environmental Protection Act, 1999*. S.C., 1999, c. 33. Available from: http://www.gazette.gc.ca/archives/p3/1999/g3-02203.pdf

Canada. 2000. *Canadian Environmental Protection Act, 1999: Persistence and Bioaccumulation Regulations*, P.C. 2000-348, 29 March 2000, SOR/2000-107. Available from: http://www.gazette.gc.ca/archives/p2/2000/2000-03-29/pdf/g2-13407.pdf

Canada, Dept. of the Environment, Dept. of Health. 2006. *Canadian Environmental Protection Act, 1999*: *Notice of intent to develop and implement measures to assess and manage the risks posed by certain substances to the health of Canadians and their environment*. Canada Gazette, Part I, vol. 140, no. 49, p. 4109–4117. Available from: http://www.gazette.gc.ca/archives/p1/2006/2006-12-09/pdf/g1-14049.pdf

Canada, Dept. of the Environment, Dept. of Health. 2009. *Canadian Environmental Protection Act, 1999: Notice with respect to Batch 8 Challenge substances.* Canada Gazette, Part I, vol. 143, no. 5. Available from: http://www.gazette.gc.ca/rp-pr/p1/2009/2009-01-31/pdf/g1-14305.pdf

CanTox Inc. 1996. Assessment of human exposure to sodium nitrilotriacetate monohydrate from the use of various industrial/institutional and consumer products. Mississauga (ON): Monsanto Company.

[CATABOL] Probabilistic assessment of biodegradability and metabolic pathways [Computer Model]. ©2004–2008. Version 5.10.2. Bourgas (BG): Bourgas Prof. Assen Zlatarov University, Laboratory of Mathematical Chemistry. Available from: http://oasis-lmc.org/?section=software&swid=1

Celotti L, Furlan D, Seccati L, Levis AG. 1987. Interactions of nitrilotriacetic acid (NTA) with Cr (VI) compounds in the induction of gene mutations in cultured mammalian cells. Mutat Res 190: 35–39.

Celotti L, Furlan D, Ferraro P, Levis AG. 1988. DNA damage and repair induced *in vitro* by nitrilotriacetic acid (NTA) in human lymphocytes. Mutat Res 209: 149–154.

Chu I, Becking GC, Villeneuve DC, Viau A. 1978. Metabolism of nitrilotriacetic acid (NTA) in the mouse. Bull Environ Contam Toxicol 19: 417–422.

[CNS] Cosmetic Notification System [Proprietary Database]. 2009. Available from Health Canada, Cosmetics Division.

Coker JN. 1972. Sodium nitrilotriacetate—Two-step alkaline process. Ind Eng Chem Prod Res Dev 11(4): 450–454.

[ConsExpo] Consumer Exposure Model [Internet]. 2006. Version 4.1. Bilthoven (NL): Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment). Available from: http://www.rivm.nl/en/healthanddisease/productsafety/ConsExpo.jsp#tcm:13–42840

Costa R, Russo A, Zordan M, Pacchierotti A, Tavella A, Levis AG. 1988a. Nitrilotriacetic acid (NTA) induces aneuploidy in *Drosophila* and mouse germ-line cells. Environ Mol Mutagen 12: 397–407.

Costa R, Strolego G, Levis AG. 1988b. Mutagenicity of lead chromate in *Drosophila melanogaster* in the presence of nitrilotriacetic acid (NTA). Mutat Res 204: 257–261.

Crebelli R, Bellincampi D, Conti G, Conti L, Morpurgo G, Carere A. 1986. A comparative study on selected chemical carcinogens for chromosome malsegregation, mitotic crossing-over and forward mutation induction in *Aspergillus nidulans*. Mutat Res 172: 139–149.

Crosbie GA, Lodi A, Miller JHM, Skellern GG. 2003. The control of nitrilotriacetic acid in edetic acid and its salts by liquid chromatography. J Pharm Biomed Anal 33: 435–440.

Deligiannis G, Piccione J, editors. 2008. 2008 Canadian chemical directory. Scarborough (ON): Camford.

Ding W-H, Wu J, Semadeni M, Reinhard M. 1999. Occurrence and behavior of wastewater indicators in the Santa Ana River and the underlying aquifers. Chemosphere 39(11): 1781–1794.

Dow Chemical Company. 1987. Food additive petition on pentasodium EDTA and trisodium EDTA. Submitted to the US Food and Drug Administration. FAP 7B4023. [cited in Lanigan and Yamarik 2002].

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon VF. 1985. Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli.* Environ Mutagen 7: 1–248.

Dunkel VC, Schechtman LM, Tu AS, Sivak A, Lubet RA, Cameron TP. 1988. Interlaboratory evaluation of the C3H/10T1/2 cell transformation assay. Environ Mol Mutagen 12: 21–31.

[ECOTOX] ECOTOXicology database [database on the Internet]. 2006. Washington (DC): US Environmental Protection Agency, Office of Research and Development, National Health and

Environmental Effects Research Laboratory, Mid-Continent Ecology Division. Available from: http://cfpub.epa.gov/ecotox

Eichinger R. 2005. Paper and pulp [Internet]. In: Ullmann's encyclopedia of industrial chemistry, online version. Available from: http://mrw.interscience.wiley.com/emrw/9783527306732/ueic/article/a18_545/current/pdf [restricted access]

Environment Canada. 2007. Guidance for conducting ecological assessments under CEPA, 1999: science resource technical series, draft module on QSARs. Reviewed draft working document. Gatineau (QC): Environment Canada, Ecological Assessment Division.

Environment Canada. 2009a. Data for Batch 8 substances collected under Canadian Environmental Protection Act, 1999, Section 71: *Notice with respect to Batch 8 Challenge substances.* Data prepared by: Environment Canada, Existing Substances Program.

Environment Canada. 2009b. Suggested approach to determining the persistence of a chemical from biodegradation data. Preliminary draft working document. Gatineau (QC): Environment Canada, Existing Substances Program.

Environment Canada. 2009c. IGETA report: CAS RN 139-13-9, 2009-04-08. Unpublished report. Gatineau (QC): Environment Canada, Ecological Assessment Division.

[EP] European Pharmacopoeia. 2001. Edetic acid. In: European pharmacopoeia. 4th ed. Strasbourg (FR): Council of Europe, European Directorate for the Quality of Medicines. p. 1097–1098.

Epstein SS, Arnold E, Andrea S, Bass W, Bishop Y. 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol Appl Pharmacol 23: 288–325.

[EQC] Equilibrium Criterion Model. 2003. Version 2.02. Peterborough (ON): Trent University, Canadian Centre for Environmental Modelling and Chemistry. Available from: http://www.trentu.ca/academic/aminss/envmodel/models/EQC2.html

[EURAR] European Union risk assessment report: CAS No.: 5064-31-3: trisodium nitrilotriacetate [Internet]. 2005. Luxembourg: Office for Official Publications of the European Communities. Final report, 2005. On the cover, European Commission Joint Research Centre. [cited 2009 Jun]. Available from: http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=ora

[EURAR] European Union risk assessment report: CAS No.: 5064-31-3: trisodium nitrilotriacetate [Internet]. 2008. Draft dated August 20, 2008. [cited 2009 Jul]. Available from: http://echa.europa.eu/doc/trd_substances/trisodium_nitrilotriacetate/rar/trd_rar_germany_nta.rtf

European Commission. 2007. Summary record. Technical committee on classification and labelling of dangerous substances; meeting on health effects of pesticides, biocides, existing chemicals, new chemicals, and general issues. Meeting at ECB Ispra, 21–24 March 2006. European Commission, Directorate-General JRC, Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau. ECBI /90/06 Rev. 8. Available from: http://ecb.jrc.ec.europa.eu/documents/Classification-Labelling/ADOPTED_SUMMARY_RECORDS/9006r8_ECB_sr_health_0306.pdf

Fischer WK, Gericke P, Schmid R. 1974. Methodenkombination zur sukzessiven Prüfung und Bewertung der biologischen Abbaubarkeit synthetischer Substanzen. Wasser Abwasser Forsch 7(Suppl. 4): 99–118.

Fukushima S, Kurata Y, Tamano S, Inoue K, Ito N. 1985. Promoting effect of trisodium nitrilotriacetate monohydrate on urinary bladder carcinogenesis in rats. Jpn J Cancer Res 76: 823–827.

Garric J, Vollat B, Nguyen DK, Bray M, Migeon B, Kosmala A. 1996. Ecotoxicological and chemical characterization of municipal wastewater treatment plant effluents. Water Sci Technol 33(6): 83–91.

Glauser J, Blagoev M, Kishi A. 2007. CEH product review: chelating agents. Menlo Park (CA): SRI Consulting. Available from: http://www.sriconsulting.com/CEH/Public/Reports/index.html [restricted access]

Gousetis C, Opgenorth H-J. 2005. Nitrilotriacetic acid [Internet]. In: Ullmann's encyclopedia of industrial chemistry, online version. Available from: http://mrw.interscience.wiley.com/emrw/9783527306732/ueic/article/a17_377/current/pdf [restricted access]

Goyer RA, Falk HL, Hogan M, Feldman DD, Richter W. 1981. Renal tumours in rats given trisodium nitrilotriacetic acid in drinking water for two years. J Natl Cancer Inst 66: 869–880.

Greenblatt M, Lijinsky W. 1974. Carcinogenesis and chronic toxicity of nitrilotriacetic acid in Swiss mice. J Natl Cancer Inst 52: 1123–1126.

Hart JR. 2005. Ethylenediaminetetraacetic acid and related chelating agents. In: Ullmann's encyclopedia of industrial chemistry, online version. Available from: http://mrw.interscience.wiley.com/emrw/9783527306732/ueic/article/a10_095/current/pdf [restricted access]

Health Canada. 1990. Nitrilotriacetic acid (NTA). Ottawa (ON): Health Canada, Safe Environments Programme. Available from: http://dsp-psd.pwgsc.gc.ca/Collection/H48-10-1-61-1990E.pdf

Health Canada. 1998. Exposure factors for assessing total daily intake of priority substances by the general population of Canada. Unpublished report. Ottawa (ON): Health Canada, Environmental Health Directorate.

Health Canada. 2007. The cosmetic ingredient hotlist – September 2009 [Internet]. Ottawa (ON): Health Canada, Consumer Product Safety. [cited 2009 Dec 14]. Available from: http://www.hc-sc.gc.ca/cps-spc/person/cosmet/info-ind-prof/_hot-list-critique/hotlist-liste-eng.php

Health Canada. 2008. Guidelines for Canadian Drinking Water Quality—Summary table. [cited 2009 Jul 22]. Ottawa (ON): Health Canada. Available from: http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/water-eau/sum_guide-res_recom/summary-sommaire-eng.pdf

[HENRYWIN] Henry's Law Constant Program for Microsoft Windows [Estimation Model]. 2008. Version 3.20. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

Hiasa Y, Kitahor Y, Konishi N, Shimoyama T. 1984. Trisodium nitrilotriacetate monohydrate: promoting effects on the development of renal tubular cell tumours in rats treated with *N*-ethyl-*N*-hydroxyethylnitrosamine. J Natl Cancer Inst 72: 483–488.

Hiasa Y, Kitahori Y, Konishi N, Shimoyama T. 1985a. Dose-related effect of trisodium-nitrilotriacetate monohydrate on renal tumourigenesis initiated with *N*-ethyl-*N*-hydroxyethyl-nitrosamine in rats. Carcinogenesis 6: 907–910.

Hiasa Y, Kitahori Y, Konishi N, Shimoyama T, Miyashiro A. 1985b. Trisodium nitrilotriacetate monohydrate: promoting effect in urinary bladder carcinogenesis in rats treated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. J Natl Cancer Inst 74: 235–239.

[HYDROWIN] Hydrolysis Rates Program for Microsoft Windows [Estimation Model]. 2008. Version 2.00. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1990. Nitrilotriacetic acid and its salts. IARC Monogr Eval Carcinog Risks Hum 48: 181–212.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1999. Nitrilotriacetic acid and its salts. IARC Monogr Eval Carcinog Risks Hum 73: 385–399.

[IJC] International Joint Commission. 1977. A report to the Great Lakes Research Advisory Board of the International Joint Commission on the health implications of NTA. Great Lakes Advisory Board. Available on request.

Kanerva RL, Francis WR, Lefever FR, Dorr T, Alden CL, Anderson RL. 1984. Renal pelvic and ureteral dilatation in male rats ingesting trisodium nitrilotriacetate. Food Chem Toxicol 22: 749–753.

Kaur G, Hasan SK, Srivastava RC. 1980. Effect of nitrilotriacetic acid (NTA) on the distribution of manganese-54 in rats. Arch Toxicol 45: 203–206.

Kiessling D, Kaluza U. 1997. Nitrilotriacetic acid: physicochemical and ecological properties. In: Swuger MJ, editor. 1997. Detergents in the environment. New York (NY): Marcel Dekker.

Kihlman BA, Sturelid S. 1970. Nitrilotriacetic acid (NTA) and chromosome breakage. Environ Mutagen Soc Newsl 3: 32–33.

Kitahori Y, Konishi N, Shimoyama T, Hiasa Y. 1985. Dose-dependent promoting effect of trisodium nitrilotriacetate monohydrate on urinary bladder carcinogenesis in Wistar rats pretreated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. Jpn J Cancer Res 76: 818–822.

Kitahori Y, Shimoyama T, Ohshima M, Matsuki H, Hashimoto H, Minami S, Konishi N, Hiasa Y. 1988. Effects of trisodium nitrilotriacetate monohydrate, nitrilotriacetic acid and ammonium chloride on urinary bladder carcinogenesis in rats pretreated with *N*-bis (2-hydroxypropyl) nitrosamine. Cancer Lett 43: 105–110.

Knepper TP. 2003. Synthetic chelating agents and compounds exhibiting complexing properties in the aquatic environment. Trends Anal Chem 22(10): 708–724.

[KOAWIN] Octanol–Air Partition Coefficient Program for Microsoft Windows [Estimation Model]. 2008. Version 1.00. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

[KOCWIN] Soil Adsorption Partition Coefficient Program for Microsoft Windows [Estimation Model]. 2008. Version 1.00. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

[KOWWIN] Octanol–Water Partition Coefficient Program for Microsoft Windows [Estimation Model]. 2008. Version 1.67. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

Kramers PGN. 1976. Mutagenicity studies with nitrilotriacetic acid (NTA) and Citrex S-5 in *Drosophila*. Mutat Res 40: 277–280.

Krari N, Allain P. 1991. Effects of three chelating agents, EDTA, NTA, and TPP, on the concentrations of elements in rat tissues. Biol Trace Elem Res 29: 125–131.

Lanfranchi G, Paglialunga S, Levis AG. 1988. Mammalian cell transformation induced by chromium(VI) compounds in the presence of nitrilotriacetic acid. J Toxicol Environ Health 24: 251–260.

Lanigan RS, Yamarik TA. 2002. Final report on the safety assessment of EDTA, calcium disodium EDTA, diammonium EDTA, dipotassium EDTA, disodium EDTA, TEA-EDTA, tetrasodium EDTA, tripotassium EDTA, trisodium EDTA, HEDTA, and trisodium HEDTA. Int J Toxicol 21(Suppl. 2): 95–142.

Lee H-B, Peart TE, Kaiser KLE. 1996. Determination of nitrilotriacetic, ethylenediaminetetraacetic and diethylenetriaminepentaacetic acids in sewage treatment plant and paper mill effluents. J Chromatogr A 738: 91–99.

Leibold E, Deckardt K, Mellert W, Potthoff-Karl B, Grundler O, Jäckh R. 2002. NTA and Fe (III) NTA: differential patterns of renal toxicity in subchronic studies. Hum Exp Toxicol 21: 445–452.

Lijinsky W, Greenblatt M, Kommineni C. 1973. Brief communication: feeding studies of nitrilotriacetatic acid and derivatives in rats. J Natl Cancer Inst 50: 1061–1063.

Loprieno N, Boncristiani G, Venier P, Montaldi A, Majone F, Bianchi V, Paglialunga S, Levis AG. 1985. Increased mutagenicity of chromium compounds by nitrilotriacetic acid. Environ Mutagen 7: 185–200.

Loveday KS, Lug MH, Resnick MA, Anderson BE, Zeiger E. 1989. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*. II. Results with 20 chemicals. Environ Mol Mutagen 13: 60–94.

Lynn JL Jr. 2009. Detergency and detergents [Internet]. In: Kirk-Othmer encyclopedia of chemical technology, online version. Available from: http://mrw.interscience.wiley.com/emrw/9780471238966/kirk/article/steabell.a01/current/pdf [restricted access]

Mahaffey KR, Goyer RA. 1972. Trisodium nitrilotriacetate in drinking water. Metabolic and renal effects in rats. Arch Environ Health 25: 271–275.

Malaiyandi M, Williams DT, O'Grady R. 1979. A national survey of nitrilotriacetic acid in Canadian drinking water. Environ Sci Technol 13(1): 59–62.

Mason JM, Valencia R, Zimmering S. 1992. Chemical mutagenesis testing in *Drosophila*: VIII. Reexamination of equivocal results. Environ Mol Mutagen 19: 227–234.

Matheson DH. 1977. Nitrilotriacetic acid (NTA) in the Canadian environment. Ottawa (ON): Environment Canada, Inland Waters Directorate, Water Quality Branch. Scientific Series No. 74. Available on request.

Matsuki H, Ozono S, Yamaguchi H, Tsunemi K, Hirao Y, Okajima E. 1992. Effects of various chemicals on the development of mouse urinary bladder tumours induced by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. J Toxicol Pathol 5: 55–60.

McFuff RE, Mord FM. 1973. Description and use of the chemical equilibrium program REDEQL2. Pasadena (CA): California Institute of Technology, W.M. Keck Laboratory of Environmental Science. Technical Report EQ-73-02. [cited in Health Canada 1990].

Merski JA. 1982. Alterations of renal tissue structure during a 30-day gavage study with nitrilotriacetate. Food Chem Toxicol 20: 433–440.

Michael WR, Wakim JM. 1971. Metabolism of nitrilotriacetic acid (NTA). Toxicol Appl Pharmacol 18: 407–416.

Michael WR, Wakim JM. 1973. Effect of trisodium nitrilotriacetate (Na_3NTA) on the metabolism of selected metal ions. Toxicol Appl Pharmacol 24: 519–529.

Millington LA, Goulding KH, Adams N. 1988. The influence of growth medium composition on the toxicity of chemicals to algae. Water Res 22: 1593–1597.

Mitchell A, Rudd CJ, Caspary WJ. 1988. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at SRI International. Environ Mol Mutagen 12: 37–101.

Modesti D, Tanzarella C, Degrassi F. 1995. Genotoxic activity of nitrilotriacetic acid in Chinese hamster cells. Mutat Res 343: 1–6.

Modler RF, Janshekar H, Inoguchi Y. 2007. CEH marketing research report: surfactants, household detergents and their raw materials. Menlo Park (CA): SRI Consulting. Available from: http://www.sriconsulting.com/CEH/Public/Reports/index.html [restricted access]

Monsanto. 1968. Toxicological investigation of: trisodium nitrilotriacetate (NTA). Unpublished report. Monsanto, Younger Laboratories. Project No. Y-68-13, dated February 2, 1968. [cited in EURAR 2008].

Montaldi A, Zentilin L, Venier P, Gola I, Bianchi V, Paglialunga S, Levis AG. 1985. Interaction of nitrilotriacetic acid with heavy metals in the induction of sister chromatid exchanges in cultured mammalian cells. Environ Mutagen 7: 381–390.

Montaldi A, Zentilin L, Zordan M, Bianchi V, Levis AG. 1987. Chromosomal effects of heavy metals (Cd, Cr, Hg, Ni and Pb) on cultured mammalian cells in the presence of nitrilotriacetic acid (NTA). Environ Toxicol Chem 14: 183–200.

Montaldi A, Mariot R, Zordan M, Paleologo M, Levis AG. 1988. Nitrilotriacetic acid (NTA) does not induce chromosomal damage in mammalian cells either *in vitro* or *in vivo*. Mutat Res 208: 95–100.

[MPBPWIN] Melting Point Boiling Point Program for Microsoft Windows [Estimation Model]. 2008. Version 1.43. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

Mueller F, Peggau J, Arif S. 2006. Special purpose cleaning formulations: auto care and industrial/institutional products. In: Showell MS, editor. Handbook of detergents. Part D: Formulations. Surfactant Science Series, Vol. 128. Boca Raton (FL): Taylor & Francis. p. 261–277.

Myers MC, Kanerva RL, Alden CL, Anderson RL. 1982. Reversibility of nephrotoxicity induced in rats by nitrilotriacetate in subchronic feeding studies. Food Chem Toxicol 20: 925–934.

Myhr BC, Caspary WJ. 1988. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. Environ Mol Mutagen 12: 103–194.

National Chemical Inventories [database on a CD-ROM]. 2008. Columbus (OH): American Chemical Society, Chemical Abstracts Service. Available from: http://www.cas.org/products/cd/nci/require.html

[NCI] National Cancer Institute (US). 1977. Bioassays of nitrilotriacetic acid (NTA) and nitrilotriacetic acid, trisodium salt, monohydrate ($Na_3NTA \cdot H_2O$) for possible carcinogenicity. Bethesda (MD): US

Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute.

Nesslany F, Simar-Meintières S, Watzinger M, Talahari I, Marzin D. 2008. Characterization of the genotoxicity of nitrilotriacetic acid. Environ Mol Mutagen 49: 439–452.

[NHW] Dept. of National Health and Welfare (CA). 1990. Present patterns and trends in infant feeding in Canada. Ottawa (ON): Department of National Health and Welfare. NHW Cat. No. H39-199/1990E. [cited in Health Canada 1998].

Nixon GA. 1971. Toxicity evaluation of trisodium nitrilotriacetate. Toxicol Appl Pharmacol 18: 398–406.

Nixon GA, Buehler EV, Niewenhuis RJ. 1972. Two-year rat feeding study with trisodium nitrilotriacetate and its calcium chelate. Toxicol Appl Pharmacol 21: 244–252.

Nolen GA, Klusman LW, Back DL, Buehler EV. 1971. Reproduction and teratology studies of trisodium nitrilotriacetate in rats and rabbits. Food Cosmet Toxicol 9: 509–518.

Nolen GA, Bohne RL, Buehler EV. 1972a. Effects of trisodium nitrilotriacetate, trisodium citrate and a trisodium nitrilotriacetate–ferric chloride mixture on cadmium and methyl mercury toxicity and teratogenicity in rats. Toxicol Appl Pharmacol 23: 238–250.

Nolen GA, Buehler EV, Geil RG, Goldenthal EI. 1972b. Effects of trisodium nitrilotriacetate on cadmium and methyl mercury toxicity and teratogenicity in rats. Toxicol Appl Pharmacol 23: 222–237.

[NPRI] National Pollutant Release Inventory [database on the Internet]. 2006. Gatineau (QC): Environment Canada. [cited 2009 May]. Available from: http://www.ec.gc.ca/pdb/querysite/query_e.cfm

[NTP] National Toxicology Program (US). 2005. Substance profiles: nitrilotriacetic acid. In: 11th report on carcinogens (RoC) [Internet]. Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. [cited 2009 Jul]. Available from: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s119nitr.pdf

[OASIS Forecast] Optimized Approach based on Structural Indices Set [Internet]. 2005. Version 1.20. Bourgas (BG): Bourgas Prof. Assen Zlatarov University, Laboratory of Mathematical Chemistry. Available from: http://oasis-lmc.org/?section=software

[PhysProp] Interactive PhysProp Database [database on the Internet]. 2006. Syracuse (NY): Syracuse Research Corporation. Available from: http://www.syrres.com/esc/physdemo.htm

Pietsch J, Schmidt W, Sacher F, Fichtner S, Brauch H-J. 1995. Pesticides and other organic micro pollutants in the river Elbe. Fresenius J Anal Chem 353: 75–82.

[PMRA] Pest Management Regulatory Agency. 2007. PMRA List of Formulants. Ottawa (ON): Health Canada, Pest Management Regulatory Agency. [cited 2009 Jul 8]. Available from: http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_decisions/reg2007-04/index-eng.php

[PMRA] Pest Management Regulatory Agency. 2009. PMRA Product Label Database [database on the Internet]. Ottawa (ON): Health Canada, Pest Management Regulatory Agency. [cited 2009 Jul 8]. Available from: http://pr-rp.pmra-arla.gc.ca/portal/page?_pageid=34,17551&_dad=portal&_schema=PORTAL

Pollack S, Ruocco S. 1981. Synergistic effect of nitrilotriacetate on iron mobilization by desferrioxamine *in vivo*. Blood 57(6): 1117–1118.

Procter & Gamble Company. 1972. New York State (Long Island Study). Mimeographed April 10, 1974. Cincinnati (OH): Procter & Gamble. [cited in Matheson 1977].

Procter & Gamble Company. 1981. Summary of Indiana Monitoring Program for NTA. Submission to US Environmental Protection Agency. Cincinnati (OH): Procter & Gamble. [cited in Anderson et al. 1985].

Procter & Gamble Company. 1982. Summary of Indiana Monitoring Program for NTA. Submission to US Environmental Protection Agency. Cincinnati (OH): Procter & Gamble. [cited in Anderson et al. 1985].

Procter & Gamble Company. 1983a. Canadian tap water monitoring for NTA, Phase I—Ottawa-Carleton and Finch, Ontario, and Phase II—Port Kells, British Columbia. Submission to the New York State Department of Environmental Conservation. Cincinnati (OH): Procter & Gamble. [cited in Anderson et al. 1985].

Procter & Gamble Company. 1983b. New York tap water monitoring for NTA, October 1981–June 1983. Submission to the New York State Department of Environmental Conservation. Cincinnati (OH): Procter & Gamble. [cited in Anderson et al. 1985].

Procter & Gamble Company. 1983c. Summary of the NTA Environmental Monitoring Program in Indiana. Submission to the New York State Department of Environmental Conservation. Cincinnati (OH): Procter & Gamble. [cited in Anderson et al. 1985].

Ramel C, Magnusson J. 1979. Chemical induction of non-disjunction in *Drosophila*. Environ Health Perspect 31: 59–66.

RCC-CCR. 2000. Hyperhaploidy–aneuploidy determination in secondary spermatocytes of mice after oral treatment with nitrilotriacetic acid (trisodium salt). Rossdorf (DE): RCC Cytotest Cell Research. Project 560100. [cited in EURAR 2008].

Richardson ML. 1992–1994. The dictionary of substances and their effects. Vols. 1–7. London (GB): Royal Society of Chemistry. Available from: http://www.rsc.org/Publishing/CurrentAwareness/DOSE/DOSESearchPage.cfm

[RIVM] Rijksinstituut voor Volksgezondheid en Milieu. 2006a. Cosmetics fact sheet: To assess the risks for the consumer. Updated version for ConsExpo 4 [Internet]. [cited 2009 Aug 31]. Bilthoven (NL): RIVM (National Institute for Public Health and the Environment). RIVM Report 320104001/2006. Available from: http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf

[RIVM] Rijksinstituut voor Volksgezondheid en Milieu. 2006b. Cleaning products fact sheet: To assess the risks for the consumer. Updated version for ConsExpo 4 [Internet]. [cited 2009 Aug 31]. Bilthoven (NL): RIVM (National Institute for Public Health and the Environment). RIVM Report 320104003/2006. Available from: http://www.rivm.nl/bibliotheek/rapporten/320104003.pdf

[RIVM] Rijksinstituut voor Volksgezondheid en Milieu. 2006c. Pest control products fact sheet: To assess the risks for the consumer. Updated version for ConsExpo 4 [Internet]. [cited 2009 Aug 31]. Bilthoven (NL): RIVM (National Institute for Public Health and the Environment). RIVM Report 320005002/2006. Available from: http://www.rivm.nl/bibliotheek/rapporten/320005002.pdf

Robbiano L, Carrozzino R, Puglia CP, Corbu C, Brambilla G. 1999. Correlation between induction of DNA fragmentation and micronuclei formation in kidney cells from rats and humans and tissue-specific carcinogenic activity. Toxicol Appl Pharmacol 161: 153–159.

Robbiano L, Carrozzino R, Bacigalupo M, Corbu C, Brambilla G. 2002. Correlation between induction of DNA fragmentation in urinary bladder cells from rats and humans and tissue-specific carcinogenic activity. Toxicology 179: 115–128.

Russo A, Pacchierotti F, Bassani B, Levis AG. 1989. Lack of induction of somatic aneuploidy in the mouse by nitrilotriacetic acid (NTA). Mutat Res 226: 111–114.

Schmidt CK, Fleig M, Sacher F, Brauch H-J. 2004. Occurrence of aminopolycarboxylates in the aquatic environment of Germany. Environ Pollut 131: 107–124.

[SCJGSARA] S.C. Johnson Global Safety Assessment & Regulatory Affairs. 2009. Material safety data sheet: Scrubbing Bubbles® Bathroom Cleaner – Fresh Clean [Internet]. Racine (WI): S.C. Johnson and Son, Limited. [cited 2009 Aug 26]. Available from: http://www.scjohnson.ca/msds/Scrubbing%20Bubbles%20Bathroom%20Cleaner%20-Fresh%20Clean%20Jan%202009.pdf

Stabel H-H. 1998. Chemical composition and drinking water quality of the water from Lake Constance. Arch Hydrobiol Spec Issues Adv Limnol 53: 13–30.

Sturm RN, Payne AG. 1973. Environmental testing of trisodium nitrilotriacetate: bioassays for aquatic safety and algal stimulation. P. 403 – 424 In Glass GE (Ed.). Bioassay Techniques and Environmental Chemistry. Ann Arbor Sci. Publ. Inc., Ann Arbor MI.

Suresh B, Kishi A. 2006. CEH product review: hydrogen cyanide. Menlo Park (CA): SRI Consulting. Available from: http://www.sriconsulting.com/CEH/Public/Reports/index.html [restricted access]

Tjälve H. 1972. A study of the distribution and teratogenicity of nitrilotriacetic acid (NTA) in mice. Toxicol Appl Pharmacol 23: 216–221.

[US EPA] US Environmental Protection Agency. 1980. Final report: NTA. Unpublished report. Washington (DC): US Environmental Protection Agency, Office of Pesticides and Toxic Substances, Assessment Division.

[USP] United States Pharmacopeia. 2000a. Edetate calcium disodium. In: United States Pharmacopeia 24. Rockville (MD): US Pharmacopeia. p. 622.

[USP] United States Pharmacopeia. 2000b. Edetate disodium. In: United States Pharmacopeia 24. Rockville (MD): US Pharmacopeia. p. 622–623.

[USP] United States Pharmacopeia. 2000c. Edetic acid. In: National Formulary 19. Rockville (MD): US Pharmacopeia. p. 2449–2450.

Venier P, Montini R, Zordan M, Clonfero E, Paleologo M, Levis AG. 1989. Induction of SOS response in *Escherichia coli* strain PQ37 by 16 chemical compounds and human urine extracts. Mutagenesis 4: 51–57.

Voulgaropoulos A, Tzivanakis N. 1992. Use of ion exchangers for the voltammetric determination of NTA and EDTA in natural waters. Electroanalysis 4: 647–651.

[WATERNT] Water Solubility Estimate – Fragment Methodology [Estimation Model]. 2008. Version 1.01. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

[WHO] World Health Organization. 1996. Nitrilotriacetic acid in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/30. World Health Organization, Geneva. Available from: http://www.who.int/water_sanitation_health/dwq/chemicals/en/nitrilotriaceticacid.pdf

Williams GM, Laspia MF, Dunkel VC. 1982. Reliability of the heptatocyte primary culture/DNA repair test in testing of coded carcinogens and noncarcinogens. Mutat Res 97: 359–370.

Williams GM, Mori H, McQueen CA. 1989. Structure–activity relationship in the rat hepatocyte DNA-repair test for 300 chemicals. Mutat Res 221: 263–286.

Woodiwiss CR, Walker RD, Brownridge FA. 1979. Concentrations of nitrilotriacetate and certain metals in Canadian wastewaters and streams: 1971-1975. Water Res 13: 599–612.

Woodruff RC, Mason JM, Valencia R, Zimmering S. 1985. Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. Environ Mutagen 7: 677–702.

Yalkowsky SH, Dannenfelser RM. 1992. The Aquasol database of aqueous solubility. 5th ed. Tucson (AZ): Univ. Arizona, College of Pharmacy.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. 1992. *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19: 2–141.

Zoller U, editor. 2009. Handbook of detergents. Part E: Applications. Surfactant Science Series, Vol. 141. Boca Raton (FL): Taylor & Francis.

Zordan M, Russo A, Costa R, Bianco N, Beltrame C, Levis AG. 1990. A concerted approach to the study of the aneuploidonic properties of two chelating agents (EDTA and NTA) in the germ and somatic cell lines of *Drosophila* and the mouse. Environ Mol Mutagen 15: 205–213.

Zordan M, Graf U, Singer D, Cinzia B, Valle LD, Osti M, Costa R, Levis AG. 1991. The genotoxicity of nitrilotriacetic acid (NTA) in a somatic mutation and recombination test in *Drosophila melanogaster*. Mutat Res 262: 253–261.

Appendix 1: Upper-bounding estimates of daily intake of NTA by the general population in Canada

Route of exposure	Estimated intake (µg/kg-bw per day) of NTA by various age groups							
	0–6 months[1,2,3]			**0.5–4 years[4]**	**5–11 years[5]**	**12–19 years[6]**	**20–59 years[7]**	**60+ years[8]**
	Breast fed	**Formula fed**	**Not formula fed**					
Ambient air[9]	NA			NA	NA	NA	NA	NA
Indoor air[10]	NA			NA	NA	NA	NA	NA
Drinking water[11]	NA	2.18	0.82	0.92	0.72	0.41	0.43	0.45
Food and beverages[12]	NA	NA	NA	NA	NA	NA	NA	NA
Soil[13]	NA			NA	NA	NA	NA	NA
Total intake	NA	2.18	0.82	0.92	0.72	0.41	0.43	0.45

Abbreviation: NA, not available.

1 No data on the concentration of NTA in human breast milk were located.

2 Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (Health Canada 1998).

3 For exclusively formula-fed infants, intake from water is synonymous with intake from food. The concentration of NTA in water used to reconstitute formula was based on available data. No data on the concentration of NTA in formula were identified in Canada. Approximately 50% of non-formula-fed infants are introduced to solid foods by 4 months of age and 90% by 6 months of age (NHW 1990).

4 Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada 1998).

5 Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada 1998).

6 Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

7 Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

8 Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

9 No data on the concentration of NTA in ambient air were located.

10 No data on the concentration of NTA in indoor air were located.

11 NTA has been detected in samples of municipally treated drinking water in Canada during two nationwide surveys (Matheson 1977; Malaiyandi et al. 1979). The maximum concentration, 20.4 µg/L, reported by Malaiyandi and co-workers, was used to estimate exposure.

12 No data on the concentration of NTA in food and beverages were located.

13 No data on the concentration of NTA in soil were located.

Appendix 2: ConsExpo sample calculation scenarios

Scenarios for consumer products that are used frequently

Consumer product scenario	**Assumptions**[1,2]	**Estimated exposure**
Face makeup pressed powder	Concentration: 0.1% (CNS 2009) **General assumptions** - Exposure frequency: 365 times/year (RIVM 2006a) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 565 cm^2 (RIVM 2006a) - Applied amount: 0.8 g (RIVM 2006a) - Uptake model: diffusion (RIVM 2006a) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[3]) (RIVM 2006a) - Exposure time: 960 min (RIVM 2006a)	**Dermal** Chronic dose = 0.00425 mg/kg-bw per day

Consumer product scenario	**Assumptions[1,2]**	**Estimated exposure**
Fragrance	Concentration: 0.1% (CNS 2009) **General assumptions** - Exposure frequency: 1095 times/year (RIVM 2006a) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to spray (RIVM 2006a) - Exposure duration: 5 min (RIVM 2006a) - Room volume: 10 m^3 (RIVM 2006a) - Ventilation rate: 2/h (RIVM 2006a) - Mass generation rate: 0.14 g/s (RIVM 2006a) - Spray duration: 0.08 min (RIVM 2006a) - Airborne fraction: 0.2 (RIVM 2006a) - Weight fraction non-volatile: 0.05 (RIVM 2006a) - Density of non-volatile: 1.5 g/cm^3 (RIVM 2006a) - Room height: 2.5 m (RIVM 2006a) - Inhalation cut-off diameter: 15 µm (RIVM 2006a) - Cloud volume: 0.0625 m^3 (RIVM 2006a) - Spraying towards exposed person (RIVM 2006a) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 200 cm^2 (RIVM 2006a) - Applied amount: 0.61 g (RIVM 2006a) - Uptake model: diffusion (RIVM 2006a) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[3]) (RIVM 2006a) - Compound concentration[4]: 0.000 789 g/cm^3 - Exposure time: 320 min (RIVM 2006a)	**Inhalation** Chronic dose = 5.24×10^{-7} mg/kg-bw per day **Dermal** Chronic dose = 0.00145 mg/kg-bw per day
Hair shampoo	Concentration: 0.1% (CNS 2009) **General assumptions** - Exposure frequency: 260 times/year (RIVM 2006a) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 1440 cm^2 (RIVM 2006a) - Applied amount: 20 g (RIVM 2006a) - Uptake fraction: 0.10 (EURAR 2008)	**Dermal** Chronic dose = 0.0201 mg/kg-bw per day

Consumer product scenario	**Assumptions[1,2]**	**Estimated exposure**
Skin cleanser for the body	Concentration: 0.1% (CNS 2009) **General assumptions** - Exposure frequency: 329 times/year (RIVM 2006a) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 17 500 cm^2 (RIVM 2006a) - Applied amount: 8.7 g (RIVM 2006a) - Uptake model: diffusion (RIVM 2006a) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[3]) (RIVM 2006a) - Exposure time: 4 min (RIVM 2006a)	**Dermal** Chronic dose = 0.000619 mg/kg-bw per day
Skin cleanser for the face	Concentration: 0.1% (CNS 2009) **General assumptions** - Exposure frequency: 730 times/year (RIVM 2006a) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 565 cm^2 (RIVM 2006a) - Applied amount: 2.5 g (RIVM 2006a) - Uptake model: diffusion (RIVM 2006a) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[3]) (RIVM 2006a) - Exposure time: 5 min (RIVM 2006a)	**Dermal** Chronic dose = 5.65×10^{-5} mg/kg-bw per day

Consumer product scenario	**Assumptions[1,2]**	**Estimated exposure**
All-purpose cleaner, spraying	Concentration: 0.0002%[5] **General assumptions** - Exposure frequency: 365 times/year (RIVM 2006b) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to spray (RIVM 2006b) - Exposure duration: 60 min (RIVM 2006b) - Room volume: 15 m^3 (RIVM 2006b) - Ventilation rate: 2.5/h (RIVM 2006b) - Mass generation rate: 0.78 g/s (RIVM 2006b) - Spray duration: 0.41 min (RIVM 2006b) - Airborne fraction: 0.2 (RIVM 2006b) - Weight fraction of non-volatile: 1.4% (EURAR 2008) - Density of non-volatile: 1.77 g/cm^3 (EURAR 2008) - Room height: 2.5 m (RIVM 2006b) - Inhalation cut-off diameter: 15 µm (RIVM 2006b) - Spraying away from exposed person (RIVM 2006b) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: constant rate (RIVM 2006b) - Contact rate: 46 mg/min (RIVM 2006b) - Release duration: 24.6 s (RIVM 2006b) - Uptake fraction: 0.10 (EURAR 2008)	Total Chronic Dose[5] = 5.33×10^{-8} mg/kg-bw per day

Consumer product scenario	**Assumptions[1,2]**	**Estimated exposure**
All-purpose cleaner, cleaning	Concentration: 0.0002%[5] **General assumptions** - Exposure frequency: 365 times/year (RIVM 2006b) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to vapour: evaporation (RIVM 2006b) - Exposure duration: 60 min (RIVM 2006b) - Room volume: 15 m^3 (RIVM 2006b) - Ventilation rate: 2.5/h (RIVM 2006b) - Applied amount: 16.2 g (RIVM 2006b) - Release area: 1.71×10^4 cm^2 (RIVM 2006b) - Application duration: 10 min (RIVM 2006b) - Molecular weight matrix: 22 g/mol (RIVM 2006b) - Mass transfer rate: 2.35×10^3 m/min (Langmuir's method) (RIVM 2006b) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006b) - Exposed area: 215 cm^2 (RIVM 2006b) - Applied amount: 0.16 g (RIVM 2006b) - Uptake fraction: 0.10 (EURAR 2008)	Total Chronic Dose[5] = 4.51×10^{-7} mg/kg-bw per day

Consumer product scenario	**Assumptions[1,2]**	**Estimated exposure**
Glass cleaner, spraying	Concentration: 0.0002%[5] **General assumptions** - Exposure frequency: 365 times/year (RIVM 2006b) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to spray (RIVM 2006b) - Exposure duration: 240 min (RIVM 2006b) - Room volume: 58 m^3 (RIVM 2006b) - Ventilation rate: 0.5/h (RIVM 2006b) - Mass generation rate: 0.78 g/s (RIVM 2006b) - Spray duration: 0.7 min (RIVM 2006b) - Airborne fraction: 0.2 (RIVM 2006b) - Weight fraction of non-volatile: 0.08 (EURAR 2008) - Density of non-volatile: 1.8 g/cm^3 (EURAR 2008) - Room height: 2.5 m (RIVM 2006b) - Inhalation cut-off diameter: 15 µm (RIVM 2006b) - Spraying away from exposed person (RIVM 2006b) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: constant rate (RIVM 2006b) - Contact rate: 46 mg/min (RIVM 2006b) - Release duration: 42 s (RIVM 2006b) - Uptake fraction: 0.10 (EURAR 2008)	Total Chronic Dose[5] = 9.08×10^{-8} mg/kg-bw per day
Glass cleaner, cleaning	Concentration: 0.0002% [6] **General assumptions** - Exposure frequency: 365 times/year (RIVM 2006b) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006b) - Exposed area: 215 cm^2 (RIVM 2006b) - Applied amount: 0.29 g (RIVM 2006b) - Uptake fraction: 0.10 (EURAR 2008)	**Dermal** Chronic dose = 8.17×10^{-7} mg/kg-bw per day

[1] An inhalation rate of 16.2 m^3/day was used in all inhalation exposure estimates (Health Canada 1998).

[2] The Cosmetic Notification System (CNS 2009) identified Na_3NTA in the personal care products shown in this table. A calculated log K_{ow} value of −2.62 was used (EURAR 2005).

[3] The greatest value in skin permeability was estimated by the Fiserova-Bergerova method (included in ConsExpo 4.1). It is expected to yield the most conservative estimate of dermal exposure when using the diffusion uptake model.

[4] The solvent used in fragrances is typically ethanol, which has a density of 0.789 g/cm^3. This was taken into consideration when determining the mass:volume ratio (i.e., compound concentration) of Na_3NTA in the consumer product fragrance.
[5] The inhalation exposure was estimated to be negligible. The total chronic dose (due to inhalation and dermal exposures) from the consumer product scenario is shown.
[6] 2009 email from Risk Management Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced.

Scenarios for products that are used occasionally

Consumer product scenarios	**Assumptions[1,2]**	**Estimated exposure**
Hair dye, semi-permanent (permanent)[3]	Concentration: 0.3% (CNS 2009) **General assumptions** - Exposure frequency: 13 (10) times/year (RIVM 2006a) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 580 cm^2 (RIVM 2006a) - Applied amount: 60 (100) g (RIVM 2006a) - Uptake model: diffusion (RIVM 2006a) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[4]) (RIVM 2006a) - Exposure time: 40 min (RIVM 2006a)	**Dermal** Acute dose = 0.000685 mg/kg-bw
Foot lotion for pedicure	Concentration: 0.1% (CNS 2009) **General assumptions** - Exposure frequency: 156 times/year[5] - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 1170 cm^2 (RIVM 2006a) - Applied amount: 1.2 g (RIVM 2006a) - Uptake model: diffusion (RIVM 2006a) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[4]) (RIVM 2006a) - Exposure time: 720 min (RIVM 2006a)	**Dermal** Acute dose = 0.00656 mg/kg-bw

Consumer product scenarios	**Assumptions**[1,2]	**Estimated exposure**
Nail polish top coat for manicure	Concentration: 0.1% (CNS 2009) **General assumptions** - Exposure frequency: 156 times/year (RIVM 2006a) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to vapour: evaporation (RIVM 2006a) - Exposure duration: 5 min (RIVM 2006a) - Room volume: 1 m^3 (RIVM 2006a) - Ventilation rate: 1/h (RIVM 2006a) - Applied amount: 0.25 g (RIVM 2006a) - Release area: 19 cm^2 (RIVM 2006a) - Application duration: 5 min (RIVM 2006a) - Molecular weight matrix: 124 g/mol (RIVM 2006a) - Mass transfer rate: 2.35×10^3 m/min (Langmuir's method) (RIVM 2006a) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006a) - Exposed area: 4 cm^2 (RIVM 2006a) - Applied amount: 0.05 g (RIVM 2006a) - Uptake model: diffusion (RIVM 2006a) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[4]) (RIVM 2006a) - Exposure time: 3.36×10^3 min (RIVM 2006a)	**Inhalation** Mean event concentration = 1.91×10^{-11} mg/m^3 **Dermal** Acute dose = 0.000121 mg/kg-bw

Consumer product scenarios	**Assumptions**[1,2]	**Estimated exposure**
Bathroom cleaner, spraying	Concentration: 1% (SCJGSARA 2009) **General assumptions** - Exposure frequency: 52 times/year (RIVM 2006b) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to spray (RIVM 2006b) - Exposure duration: 25 min (RIVM 2006b) - Room volume: 10 m^3 (RIVM 2006b) - Ventilation rate: 2/h (RIVM 2006b) - Mass generation rate: 0.39 g/s (RIVM 2006b) - Spray duration: 1.5 min (RIVM 2006b) - Airborne fraction: 0.2 (RIVM 2006b) - Weight fraction of non-volatile: 0.0622[6] - Density of non-volatile: 3 g/cm^3 (RIVM 2006b) - Room height: 2.5 m (RIVM 2006b) - Inhalation cut-off diameter: 15 µm (RIVM 2006b) - Spraying away from exposed person (RIVM 2006b) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: constant rate (RIVM 2006b) - Contact rate: 46 mg/min (RIVM 2006b) - Release duration: 90 s (RIVM 2006b) - Uptake fraction: 0.10 (EURAR 2008)	**Inhalation** Mean event concentration = 0.000229 mg/m^3 **Dermal** Acute dose = 0.000973 mg/kg-bw

Consumer product scenarios	**Assumptions**[1,2]	**Estimated exposure**
Bathroom cleaner, cleaning	Concentration: 1% (SCJGSARA 2009) **General assumptions** - Exposure frequency: 52 times/year (RIVM 2006b) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to vapour: evaporation (RIVM 2006b) - Exposure duration: 25 min (RIVM 2006b) - Room volume: 10 m^3 (RIVM 2006b) - Ventilation rate: 2/h (RIVM 2006b) - Applied amount: 30 g (RIVM 2006b) - Release area: 6.4×10^4 cm^2 (RIVM 2006b) - Application duration: 20 min (RIVM 2006b) - Molecular weight matrix: 36 g/mol (RIVM 2006b) - Mass transfer rate: 2.35×10^3 m/min (Langmuir's method) (RIVM 2006) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006b) - Exposed area: 215 cm^2 (RIVM 2006b) - Applied amount: 0.3 g (RIVM 2006b) - Uptake fraction: 0.10 (EURAR 2008)	**Inhalation** Mean event concentration = 5.77×10^{-11} mg/m^3 **Dermal** Acute dose = 0.00423 mg/kg-bw

Consumer product scenarios	**Assumptions**[1,2]	**Estimated exposure**
Insecticide, miticide	Concentration: 0.001%[7] **General assumptions** - Exposure frequency: 9 times/year (RIVM 2006c) - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to spray (RIVM 2006c) - Exposure duration: 240 min (RIVM 2006c) - Room volume: 20 m^3 (RIVM 2006c) - Ventilation rate: 0.6/h (RIVM 2006c) - Mass generation rate: 0.38 g/s (RIVM 2006c) - Spray duration: 6 min (RIVM 2006c) - Airborne fraction: 0.2 (RIVM 2006c) - Weight fraction of non-volatile: 0.01[6] - Density of non-volatile: 1.8 g/cm^3 (RIVM 2006c) - Room height: 2.5 m (RIVM 2006c) - Inhalation cut-off diameter: 15 µm (RIVM 2006c) - Spraying away from exposed person (RIVM 2006c) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: constant rate (RIVM 2006c) - Contact rate: 46 mg/min (RIVM 2006c) - Release duration: 360 s (RIVM 2006c) - Uptake fraction: 0.10 (EURAR 2008)	**Inhalation** Mean event concentration = 4.47×10^{-6} mg/m^3 **Dermal** Acute dose = 3.89×10^{-6} mg/kg-bw

Consumer product scenarios	**Assumptions**[1,2]	**Estimated exposure**
Fertilizer, liquid, mixing and loading	Concentration: 1% (BCR 2002) **General assumptions** - Exposure frequency: 1 time/week[6] - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to vapour: evaporation (RIVM 2006c) - Exposure duration: 1.33 min (RIVM 2006c) - Room volume: 1 m^3 (RIVM 2006c) - Ventilation rate: 0.6/h (RIVM 2006c) - Applied amount: 500 g (RIVM 2006c) - Release area: 20 cm^2 (RIVM 2006c) - Application duration: 1.33 min (RIVM 2006c) - Molecular weight matrix: 3000 g/mol (RIVM 2006c) - Mass transfer rate: 2.72×10^3 m/min (Langmuir's method) (RIVM 2006c) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006c) - Exposed area: 1900 cm^2 (RIVM 2006c) - Applied amount: 0.01 g (RIVM 2006c) - Uptake fraction: 0.10 (EURAR 2008)	**Inhalation** Mean event concentration = 8.68×10^{-6} mg/m^3 **Dermal** Acute dose = 0.000141 mg/kg-bw

Consumer product scenarios	**Assumptions**[1,2]	**Estimated exposure**
Fertilizer, liquid, dissolved, spraying at indoor plants	Concentration: 0.000487% (diluted[6]) (BCR 2002) **General assumptions** - Exposure frequency: 1 time/week[6] - Body weight: 70.9 kg (Health Canada 1998) **Inhalation route** - Exposure type: exposure to spray (RIVM 2006c) - Exposure duration: 240 min (RIVM 2006c) - Room volume: 20 m^3 (RIVM 2006c) - Ventilation rate: 0.6/h (RIVM 2006c) - Mass generation rate: 0.38 g/s (RIVM 2006c) - Spray duration: 6 min (RIVM 2006c) - Airborne fraction: 0.2 (RIVM 2006c) - Weight fraction of non-volatile: 0.00828[6] - Density of non-volatile: 3 g/cm^3 (RIVM 2006c) - Room height: 2.5 m (RIVM 2006c) - Inhalation cut-off diameter: 15 µm (RIVM 2006c) - Spraying away from exposed person (RIVM 2006c) - Uptake fraction: 1 **Dermal route** - Exposure type: direct dermal contact with product: constant rate (RIVM 2006c) - Exposed area: 1900 cm^3 (RIVM 2006c) - Contact rate: 46 mg/min (RIVM 2006c) - Release duration: 360 s (RIVM 2006c) - Uptake fraction: 0.10 (EURAR 2008)	**Inhalation** Mean event concentration = 1.34×10^{-6} mg/m^3 **Dermal** Acute dose = 1.9×10^{-6} mg/kg-bw
Swimming pool algicide, mixing and loading	Concentration: 0.000688% [6] **General assumptions** - Exposure frequency: 28 times/year (RIVM 2006c) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006c) - Exposed area: 215 cm^2 (RIVM 2006c) - Applied amount: 0.01 g (RIVM 2006c) - Uptake model: diffusion (RIVM 2006c) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[4]) (RIVM 2006c) - Exposure time: 3 min (RIVM 2006c)	**Dermal** Acute dose = 4.27×10^{-8} mg/kg-bw

Consumer product scenarios	**Assumptions**[1,2]	**Estimated exposure**
Swimming pool algicide, application	Concentration: 0.000688%[6] **General assumptions** - Exposure frequency: 28 times/year (RIVM 2006c) - Body weight: 70.9 kg (Health Canada 1998) **Dermal route** - Exposure type: direct dermal contact with product: instant application (RIVM 2006c) - Exposed area: 1900 cm^2 (RIVM 2006c) - Applied amount: 0.5 g (RIVM 2006c) - Uptake model: diffusion (RIVM 2006c) - Skin permeability: 4.19×10^{-5} (Fiserova-Bergerova[4]) (RIVM 2006c) - Exposure time: 5 min (RIVM 2006c)	**Dermal** Acute dose = 6.4×10^{-7} mg/kg-bw
Swimming, 0.5–4 years, post-application exposure[8]	Concentration: 5.5×10^{-9}%[9] **General assumptions** - Exposure frequency: 35 times/year (RIVM 2006c) - Body weight: 15.5 kg (Health Canada 1998) **Dermal route** - Exposure type: dermal contact with product: instant application (RIVM 2006c) - Exposed area: 5780 cm^2 (Health Canada 1998) - Applied amount: 5780 g (RIVM 2006c) - Uptake model: diffusion (RIVM 2006c) - Skin permeability: 4.19×10^{-5} cm/h (Fiserova-Bergerova[4]) (RIVM 2006c) - Release duration: 60 min (RIVM 2006c) **Oral route** - Exposure type: oral exposure to product: constant rate (RIVM 2006c) - Ingestion rate: 8300 mg/min (RIVM 2006c) - Exposure time: 60 min (RIVM 2006c) - Uptake fraction: 0.12	**Dermal** Acute dose = 8.59×10^{-10} mg/kg-bw **Oral** Acute dose = 2.12×10^{-7} mg/kg-bw

[1] An inhalation rate of 16.2 m^3/day was used in inhalation exposure estimates (Health Canada 1998).
[2] NTA was identified only in fertilizers. For all other products, which contain Na_3NTA, a calculated log K_{ow} value of −2.62 was used (EURAR 2005).
[3] Parameters for permanent hair dye are indicated in parentheses when parameters are different from those for semi-permanent hair dye.
[4] The highest value for skin permeability was estimated by the Fiserova-Bergerova method (included in ConsExpo 4.1). It is expected to yield the most conservative estimate of dermal exposure when using the diffusion uptake model.

[5] No use pattern was available for foot lotion for pedicures. According to the ConsExpo Cosmetics Fact Sheet, the frequency of using nail polish is 156 times/year (RIVM 2006a). It was assumed that Canadian female adults use foot lotion for pedicures 156 times/year.

[6] Product-specific.

[7] 2009 emails from Pest Management Regulatory Agency, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced.

[8] The age group 0.5–4 years showed the greatest potential exposure out of all age groups in a swimming scenario.

[9] Concentration of substance in an average-sized outdoor swimming pool (48 m^3) (RIVM 2006c) after application of swimming algicide (average dosage amount = 384 mL) (RIVM 2006c).

Appendix 3: Summary of health effects information for NTA and Na_3NTA

Endpoint	Lowest effect levels[1]/Results
Laboratory animals and *in vitro*	
Acute toxicity	**NTA** **Lowest oral LD_{50}** (rat) = 1470 mg/kg-bw (Richardson 1992–1994). **Other oral LD_{50}** (mouse) = 3160 mg/kg-bw (Richardson 1992–1994). **Na_3NTA** **Lowest oral LD_{50}** (monkey) = 750 mg/kg-bw (Nixon 1971). **Other oral LD_{50} values:** 1300–2220 mg/kg-bw (rat) (BASF 1985); >5000 mg/kg-bw (dog) (Nixon 1971). **Inhalation LC_{50}** (rat) = >5000 mg/m^3 (>5 mg/L) (US EPA 1980). **Dermal LC_{50}** (rabbit) = >10 000 mg/kg-bw (EURAR 2008).
Short-term repeated-dose toxicity	**NTA** **Lowest oral LOAEL** = 750 mg/kg-bw per day (1.5% NTA) based on reduced growth, increased relative kidney weight, urinary calcium, hematuria and hydronephrosis in both sexes of Charles River and Fischer 344 rats (5 or 10 per group) exposed by diet to 0% or 1.5% NTA for 4 weeks (Anderson and Kanerva 1979). [Other oral studies: Myers et al. 1982; BASF 1997a] No dermal studies were identified for NTA.

Endpoint	Lowest effect levels[1]/Results
	Na_3NTA **Lowest oral LOAEL** = 100 mg/kg-bw per day (0.1% Na_3NTA) based on increased relative kidney weight in male Sprague-Dawley rats exposed to 0%, 0.01%, 0.1% or 1% Na_3NTA (equivalent to 0, 10, 100 or 1000 mg/kg-bw per day) in drinking water for 10 weeks (marked vacuolization of renal tubules observed at highest dose). **Lowest oral LOEL** = 10 mg/kg-bw per day (0.01% Na_3NTA) based on elevated blood glucose in all treated groups, but pathogenesis of this increase was unknown (Mahaffey and Goyer 1972). **Other oral LOAEL =** 200 mg/kg-bw per day (0.73 mmol per day) based on cytoplasmic vacuolization and hyperplasia of the proximal convoluted tubule in male Sprague-Dawley rats exposed by gavage to Na_3NTA at 0, 0.73 or 7.3 mmol per day (equivalent to 0, 200 or 2000 mg/kg-bw per day) for 30 days (Merski 1982). [Other oral studies: Michael and Wakim 1973; Anderson and Kanerva 1978, 1979; Alden et al. 1981; Kanerva et al. 1984; Krari and Allain 1991; Bahnemann et al. 1998; Leibold et al. 2002] **Dermal NOAEL** = 50 mg/kg-bw per day (2.5% Na_3NTA) based on no gross or histological abnormalities observed in New Zealand rabbits (six per group, sex not specified) exposed to 0% or 2.5% Na_3NTA (equivalent to 0 or 50 mg/kg-bw per day) on intact or abraded skin for 28 days (Nixon 1971). **NTA or Na_3NTA** **Inhalation study:** Limited information based on a letter from a submitter to the US EPA: When rats, guinea pigs and monkeys (sex and strains not stated) were exposed to 0, 10, 213 or 343 mg/m^3, 6 h/day, of "non-micronized" NTA or Na_3NTA for 4 weeks, treated monkeys exhibited diarrhea; rats and guinea pigs exhibited dyspnea, but no respiratory irritation or general discomfort was observed at 343 mg/m^3 of NTA or Na_3NTA. When male albino rats were exposed to 0, 2, 20, 200 or 2000 mg/m^3 of micronized NTA or Na_3NTA, 6 h/day for 4 days, respiratory, nasal and eye irritation were reported at 2000 mg/m^3 of NTA or Na_3NTA (US EPA 1980).
Subchronic toxicity	**NTA** **Lowest oral LOAEL** = 710 mg/kg-bw per day (1% NTA) based on hyperplasias of the urinary bladder epithelia in male Wistar rats (15–20 per group) exposed by diet to 0% or 1% NTA for 28 weeks (based on the NTA-only treatment data from a two-stage initiation and promotion study) (Kitahori et al. 1988). **Lowest oral LOEL** = 10 mg/kg-bw per day (100 mg/L) based on reduced serum potassium in male rats (strain not specified) exposed to 0, 100, 1000 or 5000 mg/L in drinking water for 90 days (Becking and Yagminas 1978). No dermal or inhalation studies were identified for NTA.

Endpoint	Lowest effect levels[1]/Results
	Na_3NTA **Lowest oral LOAEL** = 150 mg/kg-bw per day (3000 ppm) based on a dose-related increase in simple hyperplasias of renal tubular cells (mean No. of lesions/cm^2) in the low and middle dose groups of male Wistar rats (24 per treated group) exposed to 0, 3000, 10 000 or 30 000 ppm (equivalent to 0, 150, 500 or 1500 mg/kg-bw per day) for 30 weeks in diet (based on the Na_3NTA-only treatment data from a two-stage initiation and promotion study) (Hiasa et al. 1985a). **Lowest oral LOEL** = 35 mg/kg-bw per day (0.15% Na_3NTA) based on an increase in mean zinc excretion in both sexes of Beagle dogs (dose related in treated males and non-dose related in treated females, four per group) exposed to 0%, 0.03%, 0.15% or 0.5% Na_3NTA (equivalent to 0, 7, 35 or 116 mg/kg-bw per day) in drinking water for 90 days (Budny et al. 1973). [Other oral studies: Nixon 1971; Anderson and Danylchuk 1980; Hiasa et al. 1985b; Kitahori et al. 1985] **Dermal NOAEL** = 50 mg/kg-bw per day (2.5% Na_3NTA) based on no gross or histological abnormalities observed in New Zealand rabbits (six per group, sex not specified) exposed to 0% or 2.5% Na_3NTA (equivalent to 0 or 50 mg/kg-bw per day) on intact or abraded skin for 91 days (65 treatments) (Nixon 1971). No inhalation studies were identified for Na_3NTA. **NTA or Na_3NTA** Several two-stage tumour initiation and promotion studies were identified for NTA or Na_3NTA. In these studies, test animals were first administered various types of nitrosamines orally. Thereafter, NTA or Na_3NTA was administered orally to the nitrosamine-treated animals to test for tumour promotion effect. Non-pretreatment animal control groups (NTA or Na_3NTA only) were also employed. **Oral LOAEL (**kidney tumour promotion effect) = 150 mg/kg-bw per day (3000 ppm Na_3NTA) based on kidney tumour development promotion effect of Na_3NTA was observed in male Wistar rats (24 per treated group) exposed in diet to *N*-ethyl-*N*-hydroxyl nitrosamine (NEELA) at 1000 ppm for 2 weeks and later to Na_3NTA for 30 weeks (tumour incidence: 0%, 16%, 22%, 100% and 100% in control, NEELA only, and NEELA plus 3000, 10 000 and 30 000 ppm Na_3NTA groups, respectively). A dose-related increase of simple hyperplasias in the Na_3NTA-treated groups (with or without initiation) was also reported (Hiasa et al. 1985a). [Other oral studies: Lijinsky et al. 1973; Greenblatt and Lijinsky 1974; Hiasa et al. 1984, 1985b; Fukushima et al. 1985; Kitahori et al. 1985, 1988; Matsuki et al. 1992]

Endpoint	Lowest effect levels[1]/Results
Chronic toxicity/ carcinogenicity	**NTA or Na_3NTA** **Oral carcinogenicity in rats:** Groups of 50 Fischer 344 rats per sex (20 per sex in control group) were exposed to diet containing **a)** NTA at 0, 7500 or 15 000 ppm (equivalent to 0, 375 or 750 mg/kg-bw per day) or **b)** the same dietary concentrations of Na_3NTA (equivalent to 0, 375 or 750 mg/kg-bw per day) for 18 months followed by a 6-month recovery period. **a)** In the NTA groups, significantly increased incidences of urinary tract tumours (mainly tubular cell adenomas and carcinomas of the kidney) were observed in males at the high dose (0/20, 1/49 and 7/48, respectively; $p = 0.006$ for linear trend). High-dose females developed transitional and squamous cell carcinomas of the urinary bladder (0/18, 2/45 and 12/48, respectively; $p < 0.001$ for linear trend) and pheochromocytomas of the adrenal gland (1/20, 0/50 and 14/48, respectively; $p < 0.001$). A significant increase in the incidence of liver adenomas in treated females was observed at the middle and high doses (2/15, 8/49 and 22/49, respectively; $p = 0.001$ for linear trend). **Non-neoplastic oral LOAEL** = 375 mg/kg-bw per day as NTA (7500 ppm) based on high incidences of chronic inflammation in the kidneys, hyperplastic lesions of the urinary system and a dose-dependent decrease in body weight gain in both sexes at all treated doses. **b)** In the Na_3NTA groups, a non-significant increase in urinary system tumours was observed. In females, a transitional cell carcinoma and a papilloma of urinary bladder at the high dose and one squamous cell and three transitional cell carcinomas at the low dose were observed. A tubular cell adenoma of the kidney and a papilloma of the ureter were observed in two different low-dose males. A dose-related decrease in body weight gain was observed in both sexes. Epithelial hyperplasia of the urinary tract occurred in the treated animals only (NCI 1977).

Endpoint	Lowest effect levels[1]/Results
	Oral carcinogenicity in mice: B6C3F1 mice (50 per sex per group and 20 controls per sex) were exposed to **a)** NTA at 0, 7500 or 15 000 ppm (equivalent to 0, 975 or 1950 mg/kg-bw per day) or **b)** Na_3NTA at 0, 2500 or 5000 ppm (equivalent to 0, 325 or 650 mg/kg-bw per day) in diet for 18 months followed by a 3-month recovery period. **a)** In the NTA groups, a significant increase in the incidence of renal tumours (mostly tubular cell adenocarcinomas) was observed in treated males (0/20, 5/49 and 24/44, respectively; $p < 0.001$) and in the high-dose females (0/20, 0/39 and 4/50, respectively; $p = 0.041$ for linear trend). **Non-neoplastic oral LOAEL** = 975 mg/kg-bw per day as NTA (7500 ppm) based on reduced body weight gain in both female treated groups and in high-dose males. Non-neoplastic lesions, such as hydronephrosis, tubular degeneration and inflammation of the kidney, were found mostly in the high dose groups. Transitional epithelial hyperplasia was observed in the ureter of one low-dose male and in the renal pelvis of one high-dose female. **b)** In the Na_3NTA groups, a dose-related increase in the incidence of hematopoietic tumours was observed in males (0/20, 4/47 and 9/50, respectively; $p = 0.015$); the tumour incidence was also increased in females, but without a clear dose–response relationship (3/18, 7/47 and 4/47, respectively). No urinary tract tumours were reported. A dose-related decrease in body weight gain was seen in both sexes. Hydronephrosis, the only non-neoplastic lesion, occurred in one low-dose animal of each sex and in 28 males and 30 females at the high dose level (NCI 1977).

Endpoint	Lowest effect levels[1]/Results
	Other diet studies in rats: Groups of Fischer 344 rats (24 per sex) received diet containing Na_3NTA at 0, 200, 2000 or 20 000 ppm (equivalent to 0, 10, 100 or 1000 mg/kg-bw per day) for 104 weeks. At the high dose, tubular cell adenomas and adenocarcinomas were seen in the kidney of 4/24 males and 4/24 females ($p = 0.004$ and 0.003, respectively), and transitional cell carcinomas developed in the ureters of 8/24 males and 6/24 females ($p < 0.001$), in the renal pelvis of 4/24 males ($p = 0.003$) and in the urinary bladder of 5/24 females ($p = 0.001$). One mid-dose female developed a papilloma of the urinary bladder. No tumours were observed in the control groups. **Non-neoplastic oral LOEL** = 10 mg/kg-bw per day as Na_3NTA (200 ppm) based on marginally increased incidences of transitional cell hyperplasia seen in the renal pelvis, ureter and urinary bladder of both sexes of animals. **Non-neoplastic oral LOAEL** = 100 mg/kg-bw per day as Na_3NTA (2000 ppm) based on increased numbers of transitional cell hyperplasia in the renal pelvis, the ureter and the urinary bladder of males and females. Body weight gain and survival rate were decreased in high-dose males, and hydronephrosis was evident only at the high dose level in both sexes (NCI 1977). Charles River CD rats (50 per sex per group) were exposed to 0%, 0.03%, 0.15% or 0.5% Na_3NTA (equivalent to 0, 19, 97 or 322 mg/kg-bw per day) in the diet for 24 months. Similar incidence rates of various types of tumours were reported in all groups (including controls) and were not considered to be treatment related. **Non-neoplastic oral LOAEL** = 97 mg/kg-bw per day (0.15% Na_3NTA) based on an increase in the incidence and severity of nephritis and nephrosis observed at 0.15% and 0.5% of Na_3NTA during the course of the study. At 6 months, mild nephrosis, consisting of hydropic degeneration of tubule cells and minor tubule dilatation, was observed in animals exposed to 0.15% Na_3NTA. An increased concentration of zinc in the urine was also noted at all doses except the low dose at several time points during the study (Nixon et al. 1972). **Drinking-water study in rats:** Groups of 196 male Sprague-Dawley rats were exposed to 0% or 0.1% Na_3NTA (equivalent to 0 or 100 mg/kg-bw per day) in their drinking water for 2 years. A significant increase in renal adenomas and adenocarcinomas (5/186 in controls and 29/183 in treatment groups, respectively; $p < 0.01$) was observed in treated animals. Overall incidences of renal tubular cell hyperplasia and nephritis were similar in the treated and control groups (reaching 85% of animals), but severe-grade renal tubular hyperplasia was significantly increased in the treated group (44/186 controls vs. 67/183 treated; $p < 0.01$) **Non-neoplastic oral LOAEL** = 100 mg/kg-bw per day (0.1% Na_3NTA) based on a higher incidence of severe-grade renal tubular hyperplasia in treated male rats compared with controls (Goyer et al. 1981). No chronic inhalation studies were identified for NTA. No chronic dermal or inhalation studies were identified for Na_3NTA.

Endpoint	Lowest effect levels[1]/Results
Genotoxicity and related endpoints: *in vivo*	**Gene mutation and recombination** **NTA** **Negative:** Sex-linked recessive lethal mutation (SLRL) in germ cells of *Drosophila melanogaster* orally dosed for 3 days or single injection (Kramers 1976; Woodruff et al. 1985; Mason et al. 1992). **Negative:** Dominant lethal assay in Swiss mice, oral (1 g/kg-bw per day for 5 days) or single intraperitoneal injection of 125 mg/kg-bw (Epstein et al. 1972). **Na_3NTA** **Negative:** SLRL in germ cells of *Drosophila melanogaster* (oral at 50 mmol/L for 3 days) (Costa et al. 1988b). **Positive:** Somatic mutation and recombination test (SMART) in *Drosophila melanogaster* orally dosed at 5–50 mmol/L for 24–42 h (Zordan et al. 1990, 1991).
	DNA damage **NTA** **Positive:** DNA fragmentation in kidney and urinary bladder cells at single oral dose of 1000 or 2000 mg/kg-bw in male Sprague-Dawley rats (Nesslany et al. 2008). **Positive:** DNA fragmentation and micronucleus formation in kidney cells (oral dose of 490 mg/kg-bw per day for 3 days) and in urinary bladder cells (oral single administration of 490 or 735 mg/kg-bw) in male Sprague-Dawley rats (Robbiano et al. 1999, 2002). **Na_3NTA** **Negative:** Micronuclei formation in bone marrow cells after single intraperitoneal administration of 200–400 mg/kg-bw in Swiss mice (Montaldi et al. 1988) and in bone marrow cells after oral dose of 500–2000 mg/kg-bw per day in male NMRI mice (BASF 2004).

Endpoint	Lowest effect levels[1]/Results
	Chromosome damage: aneuploidy induction **NTA** **Positive:** In germ cells of male mice (C57BL/Cne × C3H/Cne F_1 strain) after single intraperitoneal administration of 275 mg/kg-bw (Costa et al. 1988a). **Positive:** In germ cells of *Drosophila melanogaster* at an oral dose of 4000 ppm (Ramel and Magnusson 1979). **Na_3NTA** **Positive:** In germ cells of *Drosophila melanogaster* at an oral dose of 50 mmol/L for 3 days (Costa et al. 1988a). **Positive:** Increased spermatocyte hyperhaploidy frequencies in Charles River mice after single intraperitoneal administration of 275 mg/kg-bw (Zordan et al. 1990). **Negative:** In bone marrow cells of male mice (C57BL/Cne × C3H/Cne F_1 strain) after single intraperitoneal administration of 275 mg/kg-bw (Russo et al. 1989). **Negative:** In spermatocytes of NMRI mice at oral doses of 100, 330 or 1000 mg/kg-bw (RCC-CCR 2000).
	Sister chromatid exchange (SCE) **NTA** No SCE studies were identified for NTA. **Na_3NTA** **Negative:** In bone marrow cells of male mice (C57BL/Cne × C3H/Cne F_1 strain) after single intraperitoneal administration of 275 mg/kg-bw (Russo et al. 1989).
	DNA reaction products **NTA** No DNA reaction product studies were identified for NTA. **Na_3NTA** **Negative:** No increases in the level of 8-hydroxy-2'-deoxyguanosine in male Wistar rats subjected to 0, 150 or 20 000 ppm in diet for 1 or 4 weeks (Bahnemann et al. 1998; Leibold et al. 2002).

Endpoint	Lowest effect levels[1]/Results
Genotoxicity and related endpoints: *in vitro*	**Gene mutation and recombination** **NTA** **Negative:** *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2 *uvrA*, without metabolic activation (Zeiger et al. 1992; Nesslany et al. 2008). **Positive:** Mutation at TK locus in mouse L5178Y lymphoma cells without metabolic activation (Nesslany et al. 2008). **Na_3NTA** **Negative:** *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538, with or without metabolic activation (Dunkel et al. 1985; Loprieno et al. 1985; Zeiger et al. 1992). **Negative:** Forward mutation assay in *Aspergillus nidulans* strains (diploid P1 and haploid 35) and in *Schizosaccharomyces pombe* P1, with or without metabolic activation (Loprieno et al. 1985; Crebelli et al. 1986). **Negative:** TK locus assay in mouse L5178Y lymphoma cells, with and without metabolic activation (Mitchell et al. 1988; Myhr and Caspary 1988). **Negative:** HGPRT locus assay in heteroploid Chinese hamster cells V79 (HPRT test), without metabolic activation (Celotti et al. 1987).
	DNA damage and repair **NTA** **Positive:** DNA fragmentation in rat and human urinary bladder epithelia and kidney cells, without metabolic activation; DNA strand breaks and micronucleus formation in human and rat kidney cells, without metabolic activation (Robbiano et al. 1999, 2002). **Positive:** Micronucleus induction in mouse lymphoma cells (L5178Y, CTLL-2 and CTLL-2 bcl2) and in kidney cells of male Sprague-Dawley rats, without metabolic activation (Nesslany et al. 2008). **Negative:** SOS function assay in *Escherichia coli* (PQ37 strain), without metabolic activation (Venier et al. 1989). **Na_3NTA** **Negative:** Micronucleus assay in human lymphocytes, without metabolic activation (Montaldi et al. 1987). **Negative:** Unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes, without metabolic activation (Williams et al. 1982, 1989). **Inconclusive:** UDS assay in human lymphocytes, without metabolic activation (Celotti et al. 1988).

Endpoint	Lowest effect levels[1]/Results
	Cell transformation **NTA** No cell transformation studies were identified for NTA. **Na_3NTA** **Negative:** Syrian hamster 21/C31 fibroblasts BHK, without metabolic activation (Lanfranchi et al. 1988). **Negative:** Mouse C3H/10T1/2 cells, without metabolic activation (Dunkel et al. 1988).
	Chromosomal aberration **NTA** **Negative:** Chinese hamster ovary (CHO) cells, with and without metabolic activation (Loveday et al. 1989). **Positive:** Human lymphocytes, without metabolic activation (Nesslany et al. 2008). **Na_3NTA** **Negative:** Human lymphocytes, without metabolic activation (Montaldi et al. 1987, 1988). **Weakly positive:** Chinese hamster Cl-1 cells (chromosome anomaly and micronucleus test), without metabolic activation (Modesti et al. 1995). **Inconclusive:** Rat kangaroo cells (PT K1 cells), without metabolic activation (Kihlman and Sturelid 1970).
	Sister chromatid exchange **NTA** **Negative:** CHO cells, with and without metabolic activation (Loveday et al. 1989). **Negative:** Human lymphocytes, without metabolic activation (Brat and Williams 1984). **Na_3NTA** **Negative:** CHO cells and mouse lymphoma cells (L5178Y), without metabolic activation (Montaldi et al. 1985). **Negative:** CHO cells, without metabolic activation (Brat and Williams 1984).
Reproductive toxicity	**NTA** No reproductive toxicity studies were identified for NTA.

Endpoint	Lowest effect levels[1]/Results
	Na_3NTA A diet containing 0%, 0.1% or 0.5% Na_3NTA (equivalent to 0, 90 and 450 mg/kg-bw per day) was fed to Charles River CD rats (20 per sex per group) continuously through two generations or to groups of 20 female rats only during each period of organogenesis (days 6–15 of pregnancy). **NOAEL for reproductive toxicity** = 450 mg/kg-bw per day (0.5% Na_3NTA) based on no significant differences in the conception rate, fertility and lactation rates, lactation index, average number of live-born pups per litter, number of live pups and average number of weaned pups per culled litter. **LOAEL for systemic toxicity** = 450 mg/kg-bw per day (0.5% Na_3NTA) based on mild toxicity (not further specified) (Nolen et al. 1971).
Developmental toxicity	**NTA** No developmental toxicity studies were identified for NTA. **Na_3NTA** During the course of the above-mentioned two-generation study, groups of 20 pregnant Charles River CD rats were exposed to diet containing 0%, 0.1% or 0.5% Na_3NTA (equivalent to 0, 90 and 450 mg/kg-bw per day) during days 6–15 of gestation. Ten dams of each group were sacrificed on day 13 and day 21 of gestation. **NOAEL for developmental toxicity** = 450 mg/kg-bw per day (0.5% Na_3NTA), based on no overt signs of fetotoxicity or embryotoxicity and no teratogenic effects reported. Groups of 20 female New Zealand rabbits were treated by gavage after artificial insemination with daily doses of Na_3NTA in distilled water at 0, 2.5, 25, 100 or 250 mg/kg-bw per day during days 7–16 of gestation. Animals were sacrificed on gestation day 28. **NOAEL for developmental and maternal toxicity** = 250 mg/kg-bw per day, based on lack of treatment-related effects (Nolen et al. 1971). Teratogenic effects were not observed in a study in pregnant NMRI strain albino mice (10 per group) exposed to 0% or 0.2% Na_3NTA (equivalent to 0 or 570 mg/kg-bw per day) in drinking water on days 6–18 of gestation. **NOAEL for developmental and maternal toxicity** = 570 mg/kg-bw per day (0.2% Na_3NTA), based on no significant differences in maternal weight gain and no teratogenic effects observed in treated animals (Tjälve 1972). NOTE: Na_3NTA co-administered with cadmium dichloride or methylmercuric hydroxide via drinking water to pregnant Charles River CD rats did not increase in the incidence of cadium- or mercury-induced malformations (Nolen et al. 1972a, b).

Endpoint	Lowest effect levels[1]/Results
Sensitization	**NTA** No sensitization studies were identified for NTA. **Na_3NTA** Skin sensitization (Buehler test): Guinea pigs (10 or 20 per group) were exposed to 0% or 50% Na_3NTA formulation in water. Induction treatments were on days 0, 7 and 14. No skin irritation was observed. After challenge, none of the treated or control animals showed a skin reaction (BASF 1997b).
Irritation	**NTA** No irritation studies were identified for NTA. **Na_3NTA** Eye irritation (Draize test): One eye of each animal in groups of three albino rabbits were exposed to 0 or 100 mg dry powder of Na_3NTA for 24 h. Moderate eye irritation was detected (Monsanto 1968). Eye irritation: Groups of three albino rabbits were exposed to 0% or 38% Na_3NTA solution (applied to the conjunctival sac in one eye of each animal). Mild eye irritation was noted (EURAR 2008) Respiratory tract irritation: Groups of mice (number, sex and strain not specified) were exposed to Na_3NTA aerosol for 5 min in a special test apparatus. Treated mice exhibited irritating effects; Na_3NTA was classified as "slightly irritating" at 0.22 mg/L, as "moderately irritating" at 1.09–1.41 mg/L and as "severely irritating" at 7.6 mg/L (US EPA 1980).
Humans	
Sensitization	Closed patch tests were carried out with 66 human volunteers (sex not specified) using a 1% aqueous solution of a liquid detergent containing 20% Na_3NTA (approximately 0.5 mL of test material applied on the upper arm of test subjects for a total of nine serial applications, 24 h/application, 3 times/week for 3 weeks, followed by a challenge 2 weeks later). No evidence of sensitization was noted in these volunteers (Nixon 1971).

[1] ***LC_{50}, median lethal concentration; LD_{50}, median lethal dose; LOAEL, lowest-observed-adverse-effect level; LOEL, lowest-observed-effect level; NOAEL, no-observed-adverse-effect level.***