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Nitrilotriacetic Acid (NTA)

Guideline

The maximum acceptable concentration (MAC) for nitrilotriacetic acid (NTA) in drinking water is 0.4 mg/L (400 µg/L).

Identity, Use and Sources in the Environment

Nitrilotriacetic acid (NTA) is an aminotricarboxylic acid with an empirical formula of $C_6H_9NO_6$. In the undissociated acid form, it is composed of needles or prismatic crystals. NTA has a melting point of 241.5°C; its solubility in water at 22.5°C is 1.28 mg/mL. The pH of the saturated solution is 2.3.

NTA can sequester metal ions to form water-soluble complexes; it is an important chelating agent, with many industrial applications. Because of its ability to chelate calcium and magnesium ions, the trisodium salt is used in laundry detergents as a "builder" to replace phosphates, the use of which has been restricted by legislation in some countries owing to their contribution to the eutrophication of lakes and ponds. In 1977, the amount of NTA used in detergents in Canada was 27 299 tonnes; more recent data on consumption were not identified.[1] NTA is also used extensively in the treatment of boiler water to prevent accumulation of mineral scale. It is used to a lesser extent in photography, textile manufacturing, paper and cellulose production, metal plating and cleaning operations. NTA has been proposed as a therapeutic chelating agent for manganese poisoning[2] and for the treatment of iron overloading, as it has a synergistic effect on the mobilization of iron by desferrioxamine.[3]

NTA is present in the environment primarily as a result of its release in sewage. It biodegrades readily and, under certain conditions, is broken down by photochemical and chemical reactions.[4] NTA is degraded principally by microorganisms, by carbon–nitrogen cleavage with the formation of intermediates such as iminodiacetate, glyoxylate, glycerate, glycine and ammonia;[5–7] the metabolic end products are carbon dioxide, water, ammonia and nitrate.[4] The rate of biodegradation is largely influenced by acclimatization of the microorganisms,[8,9] temperature,[10,11] dissolved oxygen concentration in water,[12] NTA concentration[13] and water hardness.[14] Most NTA–metal complexes degrade rapidly.

The half-life for biodegradation of 1 to 100 µg/L NTA in groundwater is approximately 31 hours.[15] Complete disappearance from acclimatized river water at concentrations of 5 to 50 mg/L was reported to occur in two to six days, whereas concentrations of NTA less than 5 mg/L are expected to degrade within one day.[16,17] Acclimatization of microorganisms in two lake waters resulted in the reduction of disappearance time of up to 10 mg/L NTA from six and 11 days to four and three days, respectively.[18]

Exposure

NTA is present in drinking water primarily in the form of metal complexes, rather than as the free acid. The amount of NTA complexed with metal ions is dependent on the concentrations of the metal ion, NTA^{3-} and H^+, as well as the formation constants of the various complexes.[4] Based on one model of NTA metal ion speciation, it is predicted that at a concentration of 25 ppb in river water, 50% of the NTA is complexed with Cu^{2+} ions, 34% with Ni^{2+}, 9% with Ca^{2+} and 5% with Zn^{2+}.[19]

Because the analysis of NTA requires specific and non-routine methods (see below), it is not regularly monitored in Canadian drinking water supplies. In a national survey of 70 Canadian municipalities conducted from November 1976 to February 1977, the mean concentration of NTA in drinking water was 2.82 µg/L (range <0.2 to 30.4 µg/L). The mean concentration in raw water samples was 3.88 µg/L (range <0.2 to 33.5 µg/L). Concentrations of NTA in raw and treated water samples exceeded 10 µg/L in only 14% of the locations.[20] NTA was not detected in approximately 75% of 102 wells in three communities in two Canadian provinces (detection limit 10 µg/L); NTA concentrations in the remainder of wells for which there was other evidence of pollution by sewage were between 15 and 250 µg/L.[21]

Based on the mean concentration of NTA reported in the Canadian national survey (2.82 µg/L),[20] and assuming average daily water consumption of 1.5 L, average daily intake of NTA from drinking water is 4.23 µg.

Information on concentrations of NTA in food or ambient air has not been identified. However, it is unlikely that NTA bioconcentrates in edible animal tissues, owing to its rapid biodegradation and structure. For a very small proportion of the population in households where dishes are washed with laundry detergents containing NTA, residues present on unrinsed dishes left to drip-dry may be a source of exposure. Nixon *et al*.[22] estimated the maximum intake from this source to be 0.039 mg/kg bw per day (or 2.73 mg/d for a 70-kg adult), based on what the authors considered to be exaggerated assumptions, and suggested that intake from this source more realistically approximated 0.0025 mg/kg bw per day (0.175 mg for a 70-kg adult).

Assuming that drinking water is the primary source of exposure to NTA, the mean daily intake of NTA is likely to be less than 6 µg, primarily in the form of metal complexes.

Analytical Methods and Treatment Technology

NTA concentrations in water may be determined by gas chromatographic separation with a nitrogen-specific detector. This method is suitable for detection of NTA concentrations as low as 0.0002 mg/L.[20] Other methods for determination of NTA in water include polarography, colorimetry and gas chromatography with flame ionization detection.[4]

Anderson *et al*.[4] summarized available data concerning the removal of NTA from municipal water supplies. Chlorination with 10 to 15 ppm chlorine results in 10 to 90% removal of NTA, depending upon pH, metal content, contact time, ammonia level and NTA concentration. Ozonation at ozone concentrations typically used in treatment plants can reduce concentrations of 35 to 350 µg/L NTA by more than 80% in five minutes. Activated carbon removes only a small percentage of NTA in water, because most is complexed with metals; for example, average removal of an initial concentration of 200 µg/L NTA is 12%. Anderson *et al*.[4] also reported that artificial groundwater recharge is effective in removal of NTA from water supplies, although supporting data were not presented.

In a Canadian national survey, the efficiency of removal of concentrations of NTA greater than 10 µg/L was investigated. Removal was essentially complete in one plant where chlorine disinfection was followed by potassium permanganate oxidation, whereas chlorination and chlorination combined with ozonation were less efficient.[20]

Health Effects

NTA may be beneficial in neonatal development by increasing the bioavailability of essential elements. The iron and copper content of the milk of lactating rats administered supplements of iron and copper chelates of NTA in drinking water was higher than in controls, as was the iron and copper content of tissues of the suckling pups. There were no effects on the metal content of milk or tissues following administration of supplements of zinc–NTA chelate or NTA alone to lactating rats.[23]

The kinetics and metabolism of NTA have been investigated in several species, including humans.[24–27] Absorption of NTA from the gastrointestinal tract is rapid; however, there is considerable variation among species in the proportion of NTA eliminated in the urine. In the rat, mouse and dog, elimination is primarily via the urine (70 to 95%, 96% and 69 to 80% of orally administered dose, respectively);[24–26] in the rabbit, monkey and man, most NTA is excreted in the faeces, and elimination via the urine is much less (23%, 14% and 12%, respectively).[24,27] In eight human volunteers, concentrations in blood peaked one to two hours after ingestion of 10 mg ^{14}C-NTA in gelatin capsules. Seventy-seven percent of the administered dose was present in the faeces; approximately 12% of the dose was eliminated in the urine as unchanged NTA; and less than 0.1% was exhaled as carbon dioxide. Eighty-seven percent of the absorbed quantity (i.e., 12 to 13% of the total dose) was excreted in the urine within 24 hours.[27] 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.[24–27]

NTA accumulates in bone because it forms complexes with divalent cations such as calcium; its turnover time in bone is similar to that of calcium. NTA also accumulates in the kidney; however, it has been suggested that this is an artifact associated with the retention of urine in the kidney rather than actual uptake by renal tissue.[4] In dogs administered a dose of 20 mg/kg bw by oral intubation, concentrations of NTA 72 hours after administration were highest in the bone (2 to 3 µg/g), followed by the kidney (0.43 µg/g). The concentration of NTA in the blood decreased from a peak of 16 µg/g 75 minutes after administration to 0.02 µg/g after 72 hours.[27] Deposition of NTA in the bone and kidney has been reported in other species of experimental animals; however, concentrations decreased rapidly.[25–27] Following a single oral dose of 10 mg, the concentration of NTA in the tibia of rats declined from 29 µg/g bone after one hour to 5 µg/g bone after 48 hours.[24]

There is little information regarding the toxicity of NTA in humans. Based on physical examination, blood chemistry analysis and urinalysis, no adverse health effects were reported in the metabolism study in which volunteers ingested a single dose of 10 mg NTA.[27]

NTA does not appear to be highly acutely toxic to mammals. Oral LD_{50}s in rats and mice of 1470 and 3160 mg/kg bw, respectively, have been reported.[28] The oral LD_{50} of $Na_3NTA.H_2O$ is about 2000 mg/kg bw for rodents.[4] The oral LD_{50}s for rats for the metal complexes of NTA that are commonly found in drinking water range from 810 mg/kg bw for CuNaNTA to greater than 22 500 mg/kg bw for NiNaNTA.[4]

Results of subchronic studies in which NTA has been administered orally indicate that the kidney is the target organ and that damage is dose-dependent and rapidly induced. In male Sprague-Dawley rats (nine per group) exposed to drinking water containing 0.01, 0.1 or 1% Na_3NTA (equivalent to 7, 70 and 700 mg NTA/kg bw per day)* for 10 weeks, six of the nine animals in the high dose group died by the fourth week, and the rest appeared moribund and were sacrificed. Animals in this group had marked vacuolization of renal tubules; glycosuria was also present in five of seven of these rats. Blood glucose levels were significantly elevated at all dose levels. This hyperglycaemic effect of Na_3NTA was confirmed in a study involving a second strain of rats (Charles River CD rats), in which groups of 25 males consumed drinking water containing 0.01, 0.05 or 0.1% Na_3NTA (7, 35 or 70 mg NTA/kg bw per day) for 10 weeks. Significantly elevated blood glucose levels were observed in the two higher dose groups; the blood glucose level in the 0.01% dose group was also elevated, although not significantly. There was considerable variation in the degree of hyperglycaemia among animals. The authors speculated that the hyperglycaemia may be related to a decrease in the availability of essential metals as cofactors for insulin.[29]

In a limited investigation in which two skeletally mature dogs were administered 2.5 mg/kg bw Na_3NTA in their drinking water for seven months, radial closure rates and the percentage of osteoid seams taking a fluorescent label were decreased, suggesting interference with the mineralization process.[30] It has been concluded that 8 µg NTA/g bone would probably have no demonstrable effect on bone development in the rat, based on calculations from experimental data showing that the amount of calcium combined with NTA in the bone at this level is a small fraction (0.007%) of the total calcium turned over in 24 hours.[24]

In a subchronic bioassay in which groups of weanling Sprague-Dawley rats (10 males and 10 females per group) were fed diets containing 0, 2000, 7500, 10 000 or 20 000 ppm Na_3NTA (84, 315, 420 or 840 mg NTA/kg bw per day) for 90 days, hydronephrosis was observed in 63% of the animals in the high dose group. Mild hydropic degeneration of the kidney tubular cells was reported in four of 10 male rats in the 7500 ppm dose group; two other males in this group had tubular atrophy and dilation. Rats consuming the diet containing 10 000 ppm Na_3NTA had similar but more extensive changes to the kidney. No adverse effects were observed at 2000 ppm.[31]

The chronic toxicity and tumorigenicity of NTA administered orally have been investigated in seven bioassays in rats and mice. In the earliest of these studies, groups of weanling Charles River CD rats (50 per sex per group) were fed diets containing 0.03, 0.15 or 0.5% Na_3NTA or 0.5% of the calcium chelate of NTA for two years, with sacrifices at six, 12, 18 and 24 months. A significant increase in urinary zinc was reported in the groups receiving 0.15 and 0.5% Na_3NTA and 0.5% of the calcium chelate. This dose-dependent increase in urinary zinc was accompanied by a dose-dependent increase in renal tubular cell toxicity. Mild nephrosis consisting of hydropic degeneration of tubular cells and the minor tubule was observed at six months at 0.15 and 0.5% Na_3NTA, the incidence and severity of which became more pronounced as the study continued. Renal effects at 0.5% Na_3NTA and 0.5% calcium-NTA chelate were severe. The no-observed-adverse-effect level (NOAEL) for nephrosis or nephritis in rats was considered to be 0.03% Na_3NTA, which the authors stated was equivalent to 30 mg/kg bw per day in young rats, and 15 mg/kg bw per day as they grew older (or 20 and 10 mg NTA/kg bw per day, respectively). There were no significant increases in the incidence of tumours in any of the dose groups.[22]

Greenblatt and Lijinsky[32] conducted a study in which groups of about 80 Swiss mice were administered drinking water containing 5 g NTA/L or 5 g NTA + 1 g $NaNO_2$/L for 26 weeks. The mice were killed after 37 or 38 weeks. There was an increase in the number of lung adenomas in the group receiving NTA and $NaNO_2$ in combination; this increase was not significant when the sexes were considered separately. The authors concluded that neither NTA nor the combination of compounds was carcinogenic to mice. In a similar study conducted in groups of 15 male and 15 female MRC rats exposed

* Unless included in the cited reference, dose conversions are based on the assumption that ingestion of 1 mg/kg in food and 1 mg/L in water by a 0.25-kg rat is equivalent to 0.6 and 0.1 mg/kg bw per day, respectively, and ingestion of 1 mg/kg in food and 1 mg/L water by a 0.025-kg mouse is equivalent to 0.12 and 0.2 mg/kg bw per day, respectively.[28] A dose of 1.0 mg/kg bw per day of $Na_3NTA.H_2O$ is considered to be equivalent to 0.7 mg NTA/kg bw per day, based on molecular weights of 191 and 275 g for NTA and $Na_3NTA.H_2O$, respectively.

for 84 weeks and killed after 104 weeks, there was no evidence of carcinogenicity or other toxic effects of NTA.[33] However, the periods of administration and observation in the mice bioassay were short, and only small numbers of animals were exposed in the rat study.

The National Cancer Institute (NCI) evaluated the results of NTA carcinogenicity studies conducted at two laboratories.[34] The statistical analysis of the results of these bioassays was incomplete; where available, however, the significance of the observed effects is specified. In the first experiment, groups of 24 male and 24 female inbred Fischer 344 rats were fed diets containing 200, 2000 or 20 000 ppm $Na_3NTA.H_2O$ (8.4, 84 or 840 mg NTA/kg bw per day) for two years. A 10 to 12% decrement in body weight was observed in the high dose group; survival was also significantly decreased in males consuming the highest dose. Enlarged and hard kidneys were also observed between 60 and 64 weeks in 59% of the male rats and in 9% of the female rats in this group. A significant increase in primary neoplasms of the urinary tract was reported in both males and females in the high dose group (incidence of 58 and 54%, respectively). The tumours included transitional cell carcinomas, atubular cell carcinomas and tubular cell adenomas of the kidney, and transitional cell carcinomas of the ureter and urinary bladder. Five males and five females in the high dose group (21% for either sex) had metastatic transitional cell carcinomas, which appeared most frequently in the lung and often in the lymph node, pancreas, adrenal gland and seminal vesicle. Most of the rats of both sexes in the high dose group (exact numbers not specified) had moderate to severe nephritis or hydronephrosis. The NOAEL in this study is considered to be 2000 ppm $Na_3NTA.H_2O$ (84 mg NTA/kg bw per day).

The second study involved both Fischer 344 rats and $B6C3F_1$ mice (50 of each sex for treated groups, 20 for the control groups). Rats were fed diets containing 7500 or 15 000 ppm of H_3NTA (450 or 900 mg/kg bw per day) or $Na_3NTA.H_2O$ (315 or 630 mg NTA/kg bw per day) for 18 months, followed by the control diet for six months. The mice were fed diets containing either 7500 or 15 000 ppm H_3NTA (900 or 1800 mg/kg bw per day) or 2500 or 5000 ppm $Na_3NTA.H_2O$ (210 or 420 mg NTA/kg bw per day) for 18 months, followed by a control diet for three months. Animals were sacrificed at the end of the control diet period.

Body weight in rats was depressed in a dose-related manner by both compounds. In rats exposed to the high dose (15 000 ppm) of H_3NTA, there was a significant increase in the incidence of a variety of neoplastic lesions of the urinary tract compared with the control group (15 and 28% in males and females, respectively, vs. 0% in either sex of controls). There was also a non-significant increase in these lesions in the group of rats exposed to the low dose (7500 ppm) of H_3NTA (2 and 4% in males and females, respectively, vs. 0% in controls). A slight increase in the incidence of neoplasms of the urinary system was observed in the rats exposed to 7500 ppm of the trisodium salt (8% in both males and females) and in the animals receiving the diet containing 15 000 ppm (4% in both males and females) compared with the control group (0%). Most of the tumours were primary epithelial in origin and included tubular cell adenomas and adenocarcinomas of the kidney and papillomas of the ureter in male rats, and transitional cell carcinomas of the bladder in females. These tumours were not present in any of the control animals and only rarely develop spontaneously in the strain tested. The authors also reported a positive dose–response relationship for the incidence of tumours of the endocrine system, although the incidence of these tumours was 10, 32 and 16% (males) and 30, 26 and 50% (females) in the controls, low and high dose groups, respectively. Hyperplasia and inflammation of the urinary tract were present more frequently in treated animals than in controls. A dose-related increase in the incidence of neoplastic nodules of the liver was also reported in female rats consuming H_3NTA. All animals treated with the sodium salt had moderate to severe nephritis; less severe nephritis was present in fewer of the control animals.

In male mice receiving the high dose of H_3NTA, and in female mice of the high and low dose groups, average body weights were depressed compared with the controls (significance not reported). The average body weights of male and female mice exposed to $Na_3NTA.H_2O$ were depressed in a dose-related manner. There was a statistically significant increase in tumours of the kidney, especially tubular cell adenocarcinomas, in males ingesting 15 000 ppm H_3NTA (52% vs. 0% in control animals). Eight percent of females in the high dose group and 10% of males receiving 7500 ppm H_3NTA also developed kidney tumours. The only significant increase in tumour incidence in mice consuming $Na_3NTA.H_2O$ was a dose-related increase in the incidence of tumours of the haematopoietic system in males (0, 8 and 18% in the 0, 2500 and 5000 ppm groups, respectively). Hydronephrosis was induced in the high dose groups of mice exposed to either compound (15 000 ppm H_3NTA or 5000 ppm $Na_3NTA.H_2O$) and the male mice ingesting 7500 ppm H_3NTA; hydronephrosis was not observed in control mice.

It is not possible to establish a NOAEL or lowest-observed-adverse-effect level (LOAEL) based on the data presented for the second study in the NCI report, owing to the inadequacy of the statistical analysis of the results.

Sprague-Dawley male albino rats of two different ages (body weights 70 and 350 g at commencement of the experiment) were exposed to drinking water containing 1000 ppm Na_3NTA (70 mg NTA/kg bw per day) for two years. At the termination of the study, there were 186 rats in the control group and 183 in the experimental group. Mortality was significantly higher in the exposed rats in the first 550 days of the study period (19.7% vs. 11.2% in the controls). The incidence of renal tumours, including renal adenomas and renal adenocarcinomas, was significantly increased in the exposed animals (15.8% vs. 2.7%). Eighty-five percent of animals (both control and experimental) that survived the study period had some degree of nephritis or renal tubular hyperplasia. The incidence and severity of nephritis did not appear to be related to NTA exposure, whereas the more severe grades of hyperplasia were seen more frequently in treated animals.[35]

Based on studies[4,36–38] of the histogenesis of renal tubular cell neoplasms associated with the ingestion of high doses of NTA in animal studies, Anderson *et al*.[4] proposed the following mechanism for their induction. The initial and persistent marker of NTA-induced renal tubular cell toxicity is the formation of cytoplasmic vacuoles of the proximal convoluted tubular epithelium. Upon continued exposure, these vacuoles proceed through a series of time-and dose-dependent hyperplasias of increasing severity leading to neoplasia.[38,39] Vacuolization alone is not sufficient to induce hyperplasia, as extensive vacuolization caused by repeated intraperitoneal doses of sucrose does not progress to hyperplasia.[4] However, concomitant with progression of vacuolization, NTA exacerbates the spontaneous nephrosis and nephritis characteristic in aging rats, and, with continued exposure, a small proportion of the damaged kidneys may develop renal tubular cell adenomas and adenocarcinomas. The damage appears to be reversible prior to the stages of adenomatous hyperplasia and neoplasia.[40]

Vacuoles result following exposure to single doses by gavage in water of 0.11 mmol NTA/kg bw (21 mg/kg bw) in male Sprague-Dawley rats but not 0.073 mmol NTA/kg bw (14 mg/kg bw). Vacuoles develop within 1.5 to six hours following a single dose of 7.3 mmol NTA/kg bw (1400 mg/kg bw) by gavage in water and may persist for up to 72 hours.[41]

The renal toxicity of NTA, which is considered to be a necessary precursor for renal tubular cell neoplasms, has been attributed to alterations in divalent cation (notably zinc and calcium) distribution in the urinary tract during urinary excretion. Urinary calcium and zinc increased and urinary magnesium decreased in weanling male Charles River rats fed 2% $Na_3NTA.H_2O$ (840 mg NTA/kg bw per day) or 1.5% H_3NTA (630 mg/kg bw per day) in the diet for five weeks; conversely, in the faeces, a decrease in zinc and an increase in magnesium were observed. The disposition of ingested manganese, iron or copper is not measurably altered by high doses of NTA.[42] Renal tubular cell toxicity is believed to be dependent on an increase in reabsorption of zinc from the plasma ultrafiltrate by tubular cells.[38]

Transitional epithelial cell tumours occur only at doses of NTA higher than those that induce renal tubular cell tumours. Anderson *et al*. observed that only NTA doses that increase urinary calcium are associated with transitional epithelial cell tumours (i.e., ≥1.4 mmol/kg bw per day, or 267.4 mg/kg bw per day).[4] It has been hypothesized that at concentrations of NTA in urine that exceed the total divalent metal concentration, uncomplexed NTA extracts extracellular calcium from the transitional epithelial cells of the urinary tract faster than it can be replenished.[4] Extraction of extracellular calcium reduces cell-to-cell adhesion, causing surface erosion, which provides an inordinate and sustained mitotic stimulus, which in turn can result in neoplasia in the presence of continued exposure.[4] Reductions in extracellular calcium are hypothesized to be a significant factor in metastasis.[43]

The effect of zinc on renal tubular cell toxicity induced by NTA has been investigated. Rats (strain unspecified) were fed diets containing a constant nephrotoxic level of NTA (actual dose not specified) with one of four dietary levels (8, 14, 21 or 52 ppm, equivalent to 0.48, 0.84, 1.26 or 3.12 mg/kg bw per day) of zinc for four weeks. There was a dose-dependent increase in the incidence of severe lesions (vacuoles with nodular hyperplasia) in rats ingesting the two highest doses (21 and 52 ppm) of zinc along with NTA, whereas the lower doses of zinc with NTA produced only vacuoles without hyperplasia and two sites of simple hyperplasia in the 14 kidneys that were examined.[38] The extent of renal tubular cell vacuolization in rats (strain unspecified) induced by a dose of 7.3 mmol NTA/kg bw (1400 mg/kg bw) administered by gavage (vehicle unspecified) was increased when followed by infusion with 0.3 mmol/kg bw $ZnSO_4$ (57 mg/kg bw) compared with that induced by the same dose of NTA followed by saline infusion. However, there was no vacuolization at a lower non-toxic dose of NTA (0.073 mmol/kg bw, or 14 mg/kg bw) when followed by $ZnSO_4$ infusion. Therefore, although the availability of zinc in the plasma appears to influence the degree of vacuolization, increased availability of zinc does not appear to alter the threshold dose at which NTA induces vacuolization.[4]

High dietary doses of NTA (i.e., 10 000 ppm $Na_3NTA.H_2O$, or 420 mg NTA/kg bw per day) induced renal tubular cell tumours in 100% of "W" rats previously exposed to 1000 ppm N-ethyl-N-

hydroxyethylnitrosamine (EHEN), compared with 39% incidence at 500 ppm (21 mg NTA/kg bw per day) and 33% in control rats with EHEN-only diets. Hyperplasia of transitional cells was present in all rats administered the high dose of $Na_3NTA.H_2O$; however, no transitional cell tumours were found.[44] Doses of $Na_3NTA.H_2O$ that induced simple hyperplasia of the urinary bladder epithelium increased urinary bladder carcinogenesis initiated by N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) in Wistar rats in a dose-dependent manner.[45] Similarly, $Na_3NTA.H_2O$ enhanced urinary bladder carcinogenesis initiated by BBN in Fischer 344 rats.[46] $Na_3NTA.H_2O$ significantly increased the incidence of urinary neoplastic and pre-neoplastic lesions initiated by N-bis(2-hydroxypropyl)-nitrosamine (DHPN), whereas H_3NTA produced only a slight, insignificant increase. Treatment with $Na_3NTA.H_2O$ also increased urinary pH and sodium ion content. Simultaneous treatment with NH_4Cl reduced the enhancing activity of both compounds, as well as urinary pH and sodium ion content.[47]

The mutagenic and clastogenic potential of NTA has been investigated both *in vivo* and *in vitro*.[48–58] With the exception of several studies that involved high or prolonged dosing (where the level of NTA exceeded that of the divalent cations in the medium), the results of assays conducted to date have been largely negative, and it has been concluded that NTA is not genotoxic.[1,4] NTA increases the solubilization of some poorly soluble metal complexes and thus enhances the intracellular availability of potentially mutagenic complexes. It enhanced the induction of sister chromatid exchanges in Chinese hamster cells by insoluble salts of some heavy metals,[53,59] and some insoluble salts of chromium(VI) are mutagenic in the *Salmonella* microsome assay only in the presence of NTA or NaOH.[60]

NTA is not teratogenic when administered alone or in combination with heavy metals such as cadmium or mercury. Teratogenic effects were not observed in a study in which 10 pregnant NMRI strain albino mice consumed water containing 0.2% NTA (400 mg/kg bw per day) on days 6 through 18 of gestation, although NTA accumulated in foetal skeletons.[61] Na_3NTA was neither teratogenic nor embryotoxic in a two-generation study in Charles River CD rats fed a continuous diet containing 0.1 or 0.5% Na_3NTA (70 or 350 mg NTA/kg bw per day) or in pregnant rats fed the same diets during organogenesis.[62] Similarly, no teratogenic or embryotoxic effects were observed in rabbits ingesting up to 250 mg/kg bw per day Na_3NTA (175 mg NTA/kg bw per day) during organogenesis.[62] NTA does not enhance the teratogenic potential of cadmium or methyl mercury when they are administered simultaneously to rats in drinking water.[63,64]

Classification and Assessment

NTA is poorly absorbed in man compared with experimental animals and does not appear to be metabolized in mammalian systems. It has not been found to be teratogenic or genotoxic in studies conducted to date but has induced urinary tract tumours in rats and mice at high doses. The induction of tumours is considered to be due to cytotoxicity resulting from the chelation of divalent cations such as zinc and calcium in the urinary tract, which leads to the development of hyperplasia and neoplasia. In general, neoplasms occur only following chronic ingestion of NTA at concentrations greater than 0.5 mmol NTA/kg bw per day (100 mg/kg bw per day), whereas nephrotoxicity occurs at a lower level, between 0.05 mmol/kg bw per day (10 mg/kg bw per day) and 0.3 mmol/kg bw per day (60 mg/kg bw per day).[4]

Because NTA induces tumours only at doses higher than those that are nephrotoxic, it is classified in Group IIIB (possibly carcinogenic to man), and the acceptable daily intake (ADI) is derived by division of the LOAEL or NOAEL for nephrotoxic effects by a large uncertainty factor to account for the evidence of urinary tumour induction at high doses.

The lowest NOAEL for nephrotoxic effects was obtained in the two-year study in rats by Nixon *et al*.[22] in which an increased incidence of nephritis and nephrosis was observed in rats consuming diets containing 0.15% Na_3NTA but not 0.03% Na_3NTA (or 10 mg NTA/kg bw per day). Similar levels of NTA have induced hyperglycaemia in rats in a subchronic study.[29] Based on this NOAEL of 10 mg/kg bw per day, the ADI of NTA in drinking water is derived as follows:

$$\text{ADI} = \frac{10 \text{ mg/kg bw per day}}{1000} = 0.01 \text{ mg/kg bw per day}$$

where:

- 10 mg/kg bw per day is the lowest NOAEL for nephrotoxic effects in rats[22]
- 1000 is the uncertainty factor (x10 for interspecies variation; x10 for intraspecies variation; and x10 for the carcinogenic potential at high doses).

In view of the higher absorption of NTA in rats than in man, it should be noted that this ADI is probably conservative.

Based on the above ADI, the maximum acceptable concentration (MAC) for NTA in drinking water is derived as follows:

$$\text{MAC} = \frac{0.01 \text{ mg/kg bw per day } 70 \text{ kg bw } 0.80}{1.5 \text{ L/d}} \approx 0.4 \text{ mg/L}$$

where:

- 0.01 mg/kg bw per day is the ADI, as determined above
- 70 kg bw is the average body weight of an adult

- 0.80 is the proportion of daily intake of NTA allocated to drinking water (there are few data available on concentrations of NTA in food; however, drinking water is expected to be the primary source of intake)
- 1.5 L/d is the average daily consumption of drinking water for an adult.

Based on the rapid degradation of NTA in the environment and the non-routine nature of currently available analytical methods for this compound, it is not necessary to monitor for NTA on a regular basis in drinking water supplies, unless there is sufficient reason to suspect its presence in the source supply at levels approaching the MAC.

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