Scientific Committee on Consumer Safety

SCCS

OPINION ON

Trisodium nitrilotriacetate (NTA)

The SCCS adopted this opinion at its 9th plenary meeting on 14 December 2010

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Contact
European Commission
Health & Consumers
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

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Prof. J. Angerer
Dr. U. Bernauer
Dr. C. Chambers
Prof. G. Degen
Dr. S.C. Rastogi
Prof. V. Rogiers
Prof. T. Sanner (chairman, rapporteur)
Dr. J. van Engelen
Prof. R. Waring
Dr. I.R. White

This opinion has been subject to a commenting period of four weeks after its initial publication. All comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged.

Keywords: SCCS, scientific opinion, NTA, trisodium nitrilotriacetate, directive 76/768/ECC, CAS 5064-31-3, EC 225-768-6

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1. BACKGROUND

The Cosmetics Directive as modified by the Council and the European Parliament (2003/15/EC[1]), which is based on an opinion of the SCCNFP of September 2001 (SCCNFP/0474/01, final), stipulates that *"the use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction, of category 1, 2 and 3, under Annex I to Directive 67/548/EEC shall be prohibited. To that end the Commission shall adopt the necessary measures in accordance with the procedure referred to in Article 10(2). A substance classified in category 3 may be used in cosmetics if the substance has been evaluated by the SCCNFP and found acceptable for use in cosmetic products."*

In order to implement that provision, the Commission consulted the SCCNFP and, on 25 May 2004, the SCCNFP confirmed its opinion of 25 September 2001 (SCCNFP/0825/04). The Commission adopted Directive 2004/93/EC in order to amend accordingly Annexes II and III of the Cosmetics Directive. Subsequently, the SCCP has been consulted following each adaptation of Annex I to Council Directive 67/548/EEC[2].

On 21 August 2008 and on 15 of January 2009 the Commission adopted respectively Directives 2008/58/EC[3] and 2009/2/EC[4] amending Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances providing new classifications. The classification provided by these two Directives has been taken over by Commission Regulation 790/2009, amending EC Regulation 1272/2008, which deleted Annex I of Council Directive 67/548/EEC.

In order to consider the implementation of these new classifications, the Commission services are consulting the SCCS regarding the safety of the substances concerned by the new classification and which are not yet banned within the Cosmetics Directive.

Separate scientific requests were issued to evaluate the continued safe use of already regulated boron and perborate compounds, which were classified with specific concentration limits by the Regulation 790/2009/EC.

The aim of the present scientific request is to ensure that the same approach - as for the boron and perborate compounds – is taken for other substances classified as CMR 1B or 2 with specific concentration limits. Two other CMR substances have specific concentration limits above 0.1%.

The 2 substances in question are trisodium nitrilotriacetate EC 225-768-6 and N-methyl-2-pyrrolidone EC 212-828-1, each of which has a specific concentration limit of 5%.

- trisodium nitrilotriacetate, EC 225-768-6, is classified Carc. 2 and with a specific concentration limit of 5%. It is not used directly as ingredient, but it is present as an additive in some ingredients according to Colipa.

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1 OJ L 66, 11.03.2003, p. 26. See recital (12).
(12) "The SCCNFP stated in its opinion of 25 September 2001 that substances classified pursuant to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances(2) as carcinogenic (except substances only carcinogenic by inhalation), mutagenic or toxic for reproduction, of category 1 or 2, and substances with similar potential, must not be intentionally added to cosmetic products, and that substances classified pursuant to Directive 67/548/EEC as carcinogenic, mutagenic or toxic for reproduction, of category 3, and substances with similar potential, must not be intentionally added to cosmetic products unless it can be demonstrated that their levels do not pose a threat to the health of the consumer."
(2) OJ 196, 16.8.1967, p. 1. Directive as last amended by Commission. Directive 2001/59/EC (OJ L 225, 21.8.2001, p. 1).

2 SCCP/0888/05 and SCCP/0913/05.

3 OJ L 246, 15.09.2008, p. 1.

4 OJ L 11, 16.01.2009, p. 6

- N-methyl-2-pyrrolidone, EC 212-828-1, is classified Repr. 1B and with a specific concentration limit of 5%. As it is used in cosmetic products in a concentration below 5% and Industry awaits the approach taken by the Commission on the boron compounds, no dossier is deemed so far necessary.

2. TERMS OF REFERENCE

1. *Based on the current knowledge on the chemistry, biology, toxicology and taking into account the scientific data used for the classification purposes of trisodium nitrilotriacetate and N-methyl-2-pyrrolidone classified respectively as a carc. cat 2 and repr. 1B substance with a specific concentration limit of 5%, does the SCCS consider safe the continued use of these two substances in cosmetic products up to the specific concentration limit set out in the Commission Regulation 790/2009?*

As this mandate concerns two unrelated substances, it will be addressed in two separate opinions. The present opinion assesses the safety of trisodium nitrilotriacetate (NTA).

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

IUPAC name: Trisodium nitrilotriacetate
INCI name: Trisodium NTA

3.1.1.2. Chemical names

Nitrilotriacetic acid trisodium salt,
Trisodium 2,2',2''-nitriloacetate
Glycine, N,N-bis(carboxymethyl)-, trisodium salt (CA-Index name)

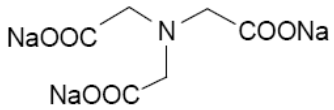
3.1.1.3. Trade names and abbreviations

NTA
NTA trisodium salt

3.1.1.4. CAS / EC number

CAS: 5064-31-3
EC: 225-768-6

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_6H_6NNa_3O_6$

3.1.2. Physical form

Colourless crystalline powder

3.1.3. Molecular weight

Molecular weight: 257.1

3.1.4. Purity, composition and substance codes

≥ 92% w/w

3.1.5.	Impurities / accompanying contaminants

< 7% water
< 3% sodium glycolate
< 2% disodium iminodi(acetate)
< 2% sodium hydroxide
< 1.5% methanamine
< 1% sodium formate

3.1.6.	Solubility

In water: about 640 g/l at 20°C

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow}: - 2.62 (calculated)

3.1.8.	Additional physical and chemical specifications

Appearance:	colourless crystalline powder
Melting point:	410 °C with decomposition above 200 °C
Boiling point:	not applicable
Density:	1.77 at 20 °C
Rel. vapour density:	/
Vapour Pressure:	/

3.1.9.	Stability

No information

3.2. Function and uses

NTA is used as a chelating and sequestering agent, and as a builder in synthetic detergents. It is also used as an eluting agent in the purification of rare earth elements, as a boiler feed water additive, in water and textile treatment, in metal plating and cleaning and in pulp and paper processing.

According to COLIPA, NTA is not used directly as cosmetic ingredient, but it is present as an additive in some ingredients.

Comment
No information on actual levels present in cosmetic products is available.

3.3. Toxicological Evaluation

The present opinion concerns trisodium nitrilotriacetate (NTA). For the evaluation of systemic effects of trisodium nitrilotriacetate (Na_3NTA, abbr. NTA) studies with administration of nitriloacetic acid (H_3NTA) or of its salts such as disodium nitrilotriacetate monohydrate ($Na_2NTA.H_2O$), and trisodium nitrilotriacetate monohydrate ($NTA.H_2O$) were considered as relevant information because these compounds are dissociated under physiological conditions (pH 7 – 9) into the sodium cations and the respective anionic species of nitrilotriacetic acid depending on the pH-dependent dissociation equilibrium of H_3NTA. Taken together, any conclusions on NTA will be derived from consideration of the overall available data base.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Monkeys: LD_{50} value of 750 mg/kg bw
Rats: LD_{50} values of 1300-1470 mg/kg bw for females and 1600-2220 mg/kg bw for males.
Dogs: LD_{50} >5000 mg/kg bw

Ref.:1

3.3.1.2. Acute dermal toxicity

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

Ref.:1

3.3.1.3. Acute inhalation toxicity

LC50 >5.0 mg/l was reported in rats in an inhalation study.

Ref.: 2

General comment
NTA has low acute toxicity by oral, dermal, and inhalation routes.

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

In a Draize skin irritation test with rabbits, unmoistened $NTA.H_2O$ of unknown purity was used (finely ground dry powder): No skin changes were noted in three rabbits within an observation period of 7 days after occlusive application of the dry powder (no information on the amount used). The dry substance was applied to the clipped intact skin and removed after 24 hours (purity and amount of substance not specified). The application sites were covered with plastic strips to avoid contamination

Ref.: 3

Mild skin irritation reversed within 5 days in a Draize test using a 25% aqueous solution of $NTA.H_2O$. The aqueous solution (no information on the amount used) was applied to the clipped intact skin of 3 albino rabbits and removed after 24 hours. The applications were covered with plastic strips to retard evaporation and avoid contamination. Findings were

negative in the first hour. Overnight there was slight to well-defined erythema with one instance of slight oedema. Redness nearly disappeared within 3 days. Mixed erythema/oedema irritation mean scores for observations 24, 48 and 72 hours after patch removal were calculated (2.3/1.3/1.0). No local signs were observed after 5 days.

Ref.: 3

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

Ref.: 4

Comment
In a 28 day rabbit repeated dose study with dermal administration (see section 3.3.5.1; ref 5) mild skin irritation was observed with 10% NTA. The NOAEL for local effects on the intact or abraded skin was 2.5%

3.3.2.2. Mucous membrane irritation

Mild eye irritation was noted in a Draize test according to OECD TG 405, when 0.1 ml of a 38% solution of NTA was applied to the conjunctival sac of 3 albino rabbits. After 24 hours, average values for redness and chemosis of the conjunctiva were 2.0 and 0.7, respectively. After 48 hours, value for conjunctival reddening was 1.0 and no chemosis was present. Conjunctival reddening was abated after 8 days. There were no findings for cornea and iris.

Ref.: 1

Moderate eye irritation was detected in a Draize test using NTA.H_2O (no data on purity): 100 mg of finely ground sample were placed in the conjunctival sac of each of 3 albino rabbits. Considerable discomfort was shown immediately upon application. Copious discharge, oedema with partial eversion of the lids, moderate redness, and sufficient congestion to mildly obscure iris details was recorded after 1 hour. The eyes were rinsed with warm isotonic saline solution after 24 hours, when discharge and oedema had already reduced. Oedema disappeared within 5 days leaving mild redness and slight corneal dullness (no information on scores) which were observed also after 7 days when the study was terminated.

Ref.: 3

Comment
Human data on local irritancy are not available. On the basis of the animal data currently available proper assessment of skin and eye irritant/corrosive properties of the substance is not possible.

The EU Risk Assessment report (ref. 1) concluded that "Based on the limited data available and taking also into account the strongly basic pH-value of a 1% aqueous solution, the current classification of R36 - Irritating to eyes - is confirmed." SCCS concur with the conclusion of the EU Risk Assessment report.

3.3.3. Skin sensitisation

Animal data

NTA (purity 92.4%) was tested in a Buehler test using guinea pigs (20 animals induced with NTA + 10 not-induced animals in control group). For induction and challenge treatments a 50% substance formulation in distilled water (0.5 ml) was used. After three induction treatments at day 0, 7 and 14 no skin irritation was noted. After challenge none of the treated or control animals showed skin reactions.

Ref.: 4

Comment by EU (ref. 1)
A positive control study (reliability check) was not performed within the above study. The most recent control study using 85% α-hexylcinnamaldehyde in the same year showed valid results. However, this study shows a significant shortcoming: a 50% formulation of NTA was used for the dermal inductions. However, the prepared formulation did not cause any sign of skin irritation, which is a prerequisite when applying this method. In consequence, the negative result of this study cannot be used for risk assessment.

Human data

Closed patch test studies were carried out with 66 volunteers using a 1% aqueous solution of a liquid detergent containing 20% NTA. The test material was applied in 0.5 ml quantities to ¾ x 1 Webril padding, placed on the upper arm of human subjects, and occluded with Elastoplast. A total of nine serial applications three times a week of three consecutive weeks, followed by a challenge two weeks after the last insult, constituted the testing procedure. Patches were removed by the subjects 24 hr after application; skin reactions were graded by trained observers after 48 and 96 hr. Challenge patches with the same concentration of the test material were placed on both the original site of insult and on an alternate site of the opposite arm, to aid the differentiation of skin fatigue and sensitization. No evidence of sensitization was noted in these volunteers (no more details on results provided).

Ref.: 5

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

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

3.3.4. Dermal / percutaneous absorption

Skin penetration of NTA has been investigated *in vitro* according to OECD 428 and OECD Guidance Document No. 28 by using radiolabelled substance (2-^{14}C-NTA), human skin preparations (split thickness skin is prepared human skin (abdomen), thickness around 400 µm) and Franz-type diffusion cells. Test substance preparations corresponding to a high (40 % NTA) and low (1% NTA) aqueous dilution were applied for a exposure time of 5 min for the 40% concentration and 6 h for the 1% concentration (5 min for the high concentration was decided due to the high pH and because "it might be taken as granted that accidental severe skin damage would greatly enhance penetration").

Each preparation (concentration) was applied onto an area of 1.0 cm^2 skin (application dose: 10 µl/cm^2); each concentration was investigated in 5 different skin samples (i.e. 5 different diffusion cells per dose). Diffusion cells were operated in a static mode under semi-occlusive conditions using tap water as receptor medium. Amounts of receptor cell fluid were taken at definite sampling times during a 24h period. At the end of the sampling period, the remaining test substance was recovered from all compartments of the diffusion cell. Radioactivity was determined in different compartments (tape strips, membrane washings, skin preparation, receptor fluid and receptor chamber washings) by liquid scintillation counting.

Results: mean total recoveries of radioactivity were 98.8% (high dose) and 101.8 % (low dose) of the applied radioactivity. The sum of radioactivity present in the skin plus radioactivity determined in the receptor fluid and receptor chamber washing was taken as the absorbed dose.

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

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

Ref.: 6

Comment
The EU Risk Assessment report (ref. 1) concluded that "For 1% NTA dilutions, an absorption rate of 1 % is taken for risk characterisation in humans based on an *in vitro* dermal absorption study. For higher NTA concentrations in solution and for animals, a higher absorption rate of 10 % is taken for risk characterisation as a default value based on considerations on the chemical structure and physico-chemical data." The SCCS concurs with this conclusion and will use the default value of 10% dermal absorption in the calculation of the MOS in the absence of adequate experimental data.

3.3.5. Repeated dose toxicity

The text of repeated dose toxicity is taken from the EU RAR report on Trisodium Nitrilotriacetate. Only the most relevant studies are reported below.

3.3.5.1. Repeated dose (28 days) oral / dermal / inhalation toxicity

Oral
Rats

H_3NTA (purity 98-99%) was administered daily to groups of 5 male and 5 female Wistar rats by gavage at dose levels of 150, 500, and 1500 mg/kg bw/day for 3 weeks. A control group received the vehicle (0.5% aqueous carboxymethylcellulose solution) only. The test protocol was not equivalent to the standard protocol for the oral 28-day study in OECD 407, since the study duration was only 3 weeks, the blood chemistry examinations were limited to urea and creatinine, haematology parameters were not examined, and histomorphologic examination was conducted on the kidney only. In addition, one male and one female of each group were perfused with formaldehyde solution and the kidneys were examined by electron microscopy with the purpose to characterize the tubular degeneration.

There was no unscheduled death during the study. Lower feed consumption was observed in high dose males from day 7 to the end of study, and in high dose females only at day 7. The consumption of feed was reduced in males of the mid and low doses in a dose-related manner; however, they were reported to be within the normal range for these animals at that age. A dose-related lower body weight and lower body weight gain were observed in treated males gaining significance at the mid and high dose levels. In the mid and high dose females, a transient reduction of the body weight and body weight gain was observed on

day 7 of the study. Diarrhoea, anogenital region smeared with urine, crust formation at the nose and piloerection were observed in high dose males and females. These groups showed a tendency to higher urea concentrations, but no clear effect on creatinine levels was seen. Urinalysis revealed non-significantly lower pH values in males in a dose-related manner and in high dose females. Increased blood and erythrocytes were detected in the urine specimens of the high dose males, whereas increased renal tubular and transitional epithelial cells and granular casts were observed in the urine of the high dose females. The mean values of absolute kidney weights were significantly higher in high dose females compared to the control group. Significantly elevated relative kidney weights were found in high dose males and females of the mid and high dose groups. Increased kidney weights relative to body weight were also seen in male rats of the mid dose group, but the increase did not gain significance. Enlarged and discoloured kidneys were observed in each one male and female of the mid and high dose groups. A white focus was noted in one kidney of a high dose male, this lesion was confirmed to be an area of severe tubular hyperplasia.

Light microscopy of the kidneys revealed tubular vacuolation, often multifocal and bilateral, in most males and females of the mid and high dose groups. Males were more affected than females, and mean severity of this lesion increased with dosage. A diffuse degeneration of the pars recta of the proximal tubule was present in three high dose males and one mid dose male. A focal degeneration of the pars recta was observed in one high dose female. The incidence of low graded basophilic (regenerating) tubules was similar between treatment and control groups, whereas the incidence of this lesion with higher severity grades increased with dosage. In addition to the focus in one high dose male confirmed to be an area of severe tubular hyperplasia, another hyperplastic lesion of the tubules was noted in a second high dose male. This lesion consisted of hyperplastic basophilic and clear cell tubules. Further findings as mononuclear cell infiltration, interstitial fibrosis, proteinaceous casts, focal glomerulopathy and calcification at the cortico-medullary junction were similarly distributed between the treatment and control groups.

Ultrastructural examinations did not show alterations of the pars recta of the proximal tubule in animals of the low and mid dose groups. A prominent vacuolation of tubular cytoplasm of a high dose male was characterised as a vacuolation and vesiculation of the rough endoplasmatic reticulum due to increased water influx. Roundish, sometimes irregular shaped vacuoles were observed in the cells of the third segment of the proximal tubule. Electron-lucent vacuoles of different sizes were regularly distributed in the cytoplasm and were limited by a singular membrane. Electron-dense structures with a diameter of 10 nm identical to the morphology of ribosomes were free or in close contact to the vacuolar membranes. Vacuolated cells appeared to have fewer cell organelles such as mitochondria and lysosomes, the content of strands of the rough endoplasmatic reticulum was markedly reduced. The observed vacuolar degeneration was regarded a consequence of acute cellular swelling which finally results in cell death. No adverse effect was observed at the dose of 150 mg/kg H_3NTA (NOAEL).

Ref.: 7

NTA.H_2O in deionised water was administered by gavage to male Sprague-Dawley rats at levels of 0, 0.73 or 7.3 mmol/kg bw/day (appr. 0, 187.6 or 1876 mg/kg bw/day) for periods up to 30 days. Two animals from each of the groups were killed 24 hours after dosing on day 9, 13, 16, 20, 23, 27 or 30. Cytoplasmic vacuolation and hyperplasia of the proximal convoluted tubules were the most prominent alterations being observed from day 9 on. The number of affected cross sections showing cytoplasmic vacuolation was much greater in the kidneys of rats given 7.3 mmol/kg NTA.H_2O than in those given the lower dose. A higher incidence of basophilic tubules and of basophilic simple tubular hyperplasia was registered in both NTA-treated groups compared to the control group. Simple tubular hyperplasia associated with vacuolation increased in a dose-related manner and was only observed in NTA-treated rats, but not in control animals. Except one case in the low dose group, the development of tubular nodular hyperplasia was observed only in high dose rats. In addition

vacuolation of epithelial cells were found in over 90% of the nodular hyperplastic lesions, the remaining were basophilic. Beginning at day 13, changes in the renal pelvic transitional epithelium were observed in high dose animals. The development of focal haemorrhage, necrosis, erosion, and hyperplasia of the epithelium of the renal pelvis were the most prominent lesions. The LOAEL for the adverse effects on the kidney was 0.73 mmol/kg bw/day (appr. 187 mg/kg bw/day).

Ref.: 8

NTA.H_2O (purity 92.4%) was administered to 14-week old male Wistar rats at dietary concentrations of 0, 150 and 20000 ppm (appr. 9 and 926 mg/kg bw/day) for 4 weeks. Subgroups of 5 animals received 150 ppm (2 groups), 20000 ppm (4 groups) or served as controls (4 groups). Animals were examined for clinical observations, food consumption, body weight (change), serum transferrin, iron, total iron-binding capacity, urinalysis on day 3 before treatment and on days 2, 4, 8, and 29 of treatment, haematology on day 30, histopathology of several organs and ultrastructural pathology of the kidneys, determinations of 8-OH-2-deoxyguanosine and of lipid peroxidation in kidneys, as well as determination of DNA-synthesis in the kidneys. In addition to standard urinalysis including the semi quantitative strip method, an extended quantitative urinalysis was performed on creatinine concentrations and enzyme activities of lactate dehydrogenase (LDH), alkaline phophatase (ALP), gamma-glutamyltransferase (GGT), and alanine aminopeptidase (AAP) and N-acetyl-ß-glucosaminidase (NAG) were measured. Urinary calcium, iron and zinc were also measured. Due to the missing haematology examinations, the incomplete clinical biochemistry, and the incomplete list of organs examined for histopathology, this study did not fulfil the requirements of current standard test protocols of the OECD 407/EEC B.7 method.

At 20000 ppm NTA group one animal died on day 28, all animals had red-brown discoloured urine from day 9 to the end of the study, some of the animals showed piloerection from day 21 on. At this dose level, food consumption was decreased up to 48% during the entire study and water consumption increased up to 102% of the controls. The body weights were significantly lower (-26%) than in the control groups. Clinical chemistry examinations did not reveal changes of the levels of iron concentrations, transferrin levels and total iron binding capacity. Quantitative measurements in the urine revealed increased activities of LDH on day 2, 4 and 8 of the study. At day 8, γ-GGT and creatinine concentrations were reduced. Increased concentrations of zinc were excreted with the urine at the end of the study, but calcium and iron excretion remained unaffected. Standard urinalysis revealed macrohematuria on day 8, increased blood and in the sediment elevated number of erythrocytes on day 4, 8, and 29. The mean specific gravity was reduced and the mean urine volume was increased (none significantly) on day 8 and 29. The urine specimens were discoloured from dark yellow to light brown and appeared cloudy on days 2, 4 and 8. Decreased amounts of urinary crystals were found on day 2, 4 and 8 and increased numbers of transitional epithelial were found on day 4 and 8 of the treatment. All animals of this dose group showed enlarged kidneys and dilation of the renal pelvis. The ureters were dilated in one animal. Microscopic lesions were seen in the kidney of all high dose animals consisting of tubular hyperplasia. These hyperplasias were characterised by tubules with large, vacuolized cells. Other changes in most or all animals of this group were basophilia, vacuolation (without hyperplasia), dilation, and calcification of tubules, interstitial nephritis, inflammation, necrosis, fibrosis and urothelial hyperplasia of the renal papilla and pelvic dilation.

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

No renal lesions except a single animal with tubular calcification and two animals with mononuclear cell infiltration were observed in the low dose group. No lesions related to the NTA treatment were observed in the liver, pancreas, and spleen. The observed lesions of other subgroups were in accordance with the described and therefore not separately reported. The NOAEL of this study was 150 ppm NTA.H_2O (9 mg/kg bw/day).

Ref.: 9

Weanling female rats (Sprague Dawley, 60 g) were fed with diet containing 0, 0.5, 0.75, 1.5 and 2% of NTA.H_2O (0, 350, 525, 1050, and 1400 mg/kg bw/day) or 0, 0.5, 0.75 and 1.5% H_3NTA (0, 350, 525, and 1050 mg/kg bw/d) for 4 weeks. The effects on growth, urine pH and urine volume were measured. The urines from the animals consuming both forms of 0.75% NTA contain insoluble material and haematuria was observed in the urines of rats at 1.5% NTA.H_2O. X-ray analyses showed that the insoluble material contained crystalline calcium chelate of NTA (CaNaNTA, not quantified). All doses of NTA.H_2O reduced growth and increased urine pH above the controls. Urine volume was reduced at 0.5% but higher doses increased the volume dose-dependently. Lower urine volume was measured at 0.5% H_3NTA and higher, but decrease did not show dose-relationship. Body weight reduction and decreased urine pH-values were seen at 0.75% and above. Related to the limited parameters under investigation, no NOAEL was estimated for both NTA forms. 0.5% (350 mg/kg bw/d) of NTA.H_2O or H_3NTA was considered as LOAEL.

Ref.: 10

Dermal

Rabbits

Two ml/kg bw/day of a 2.5% aqueous solution of NTA (≈50 mg/kg bw/day) was applied to the clipped and abraded backs (no data on exposed skin area) of 6 New Zealand rabbits (2250-2900 g) for a period of 28 days (20 treatments). An additional group of 6 animals was treated with the same dose level applied daily to intact skin for a period of 91 days (65 treatments). Two ml of a 10% solution of granular detergent (no further details given) containing 10% NTA was also applied for 5 days each week to the intact skin of 6 albino rabbits for 91 days (65 treatments) while a similar formulation containing 11% NTA was applied at the same dose level to a group of 6 animals abraded skin for 28 days (20 treatments). Animals with 2 ml/kg bw/day of water served as controls. Data on sex of animals and purity of the test substances were absent.

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

No indication for systemic effects was observed. However, systemic bioavailability of NTA is considered to be questionable, the data reported were incompliant to standard test design and therefore a N(L)OAEL for systemic toxicity cannot be derived.

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

Ref.: 5

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Oral

Rats

The reversibility of NTA-associated nephrotoxicity was investigated by comparing renal tissues from male Sprague-Dawley rats (6-8 males/group) fed nephrotoxic levels of NTA for 7 weeks with those from rats allowed 5 weeks of recovery after the 7-week exposure. Animals obtained as weanlings received 2% $NTA.H_2O$ in the diet (73 µmol/g diet, appr. 1309 mg/kg bw/day), or 1.5% H_3NTA (79 µmol/g diet, appr. 1050 mg/kg bw/day) or control diet. Throughout the study weekly weight gains and feed consumption were measured. At necropsy at the end of exposure or recovery periods the left kidney were fixed by retrograde vascular perfusion and processed for histopathologic examination. In comparison to the control group, the two forms of NTA resulted in comparable decreases in growth rate during the exposure phase of the study, these animals gained more weight during the recovery phase. Both forms induced vacuolation and hyperplasia often composed of vacuolated cells in the epithelium of the proximal convoluted tubules. These effects were noted in all of the exposed animals although the extent of damage varied. Tubular vacuolation was evident in each animal of the NTA groups, but was absent in the control groups and in the recovery groups indicating complete recovery. The total incidence of (simple) basophilic cell hyperplasia was greater in the NTA groups than in control groups after treatment and recovery period. Tubules that had a diameter more than twice of that of a normal proximal convoluted tubule were classified as tubular hyperplastic nodules of the vacuolated cell type. Nodular basophilic cell hyperplasia was only seen in treated groups and was absent in the control and recovery groups. Pelvic epithelial and subepithelial inflammation and fibrosis were observed in the groups given either form of NTA for 49 days. Mild to severe hyperplasia of the transitional epithelium (TE) in the renal pelvis occurred in 4/7 animals given diets containing $NTA.H_2O$ and moderate TE hyperplasia was observed in 1/8 rats given H_3NTA in the diet. Animals with TE hyperplasia also exhibited pelvic dilation and TE erosion, ulceration and haemorrhage. TE dysplasia, intracytoplasmic globules, and mitotic figures were also noted. Tissues from recovery animals showed no abnormal cellular morphology in the pelvic urothelium in the presence of persisting hydronephrosis. In conclusion, NTA treatment was related to vacuolation, simple and nodular hyperplasia of the vacuolated cell type and the basophilic cell type in the proximal convoluted tubules, and transitional cell erosion, ulceration, hyperplasia and dysplasia associated with hydronephrosis in the renal pelvis. Nodular hyperplasias and urothelium lesions were shown to be reversible within this model, whereas simple basophilic hyperplasia of tubules and pelvic hydronephosis persisted.

Vacuolation and basophilia of tubular cells with or without increased cell size is known to be a response to tubular injury. Lesions observed at the pelvis area are also characteristic for degenerative and regenerative response of urothelial cells. The authors discussed the higher incidence of basophilic cell hyperplasia being nonspecific and related to spontaneous age-related nephrosis. They were considered to be exacerbated by the ingestion of high doses of NTA. Although no data were reported for the acclimatisation period, it is assumed that rats were about 8 ± 2 weeks at the start of the study. At the end of study, rats were about 15 ± 2 weeks old. Therefore the role of spontaneous chronic progressive nephropathy of the old rat could not be clarified at this relatively young age and by the low number of animals (males only) examined. With respect to the renal damage, no NOAEL was established, the LOAELs were 2% $NTA.H_2O$ (appr. 1309 mg/kg bw/d) and 1.5% H_3NTA (appr. 1050 mg/kg bw/d).

Ref.: 11

In a drinking water study of 10 weeks duration NTA at either 0.01, 0.1 or 1% (w/v, appr.

10, 100, or 1000 mg/kg bw/d) was given to groups of 9 male Sprague-Dawley rats. At the end of the experiment, 24 hour urine samples were taken prior to sacrifice. Kidneys were excised and weighed. Liver, brain, and pancreas were processed for histology. There was one early death in the mid dose group and a high mortality rate in the high dose group. Six animals died within four weeks, and remaining animals appeared moribund and were killed. No difference in total body weight or weight gain was observed in 0.01 and 0.1% levels of NTA in the drinking water after ten weeks of treatment. At 0.1% NTA the relative kidney weight was elevated. Histology of the organs examined did not differ from that of control animals. In contrast, kidneys from the 1% NTA group showed marked vacuolization of renal tubules. Brain and pancreas of these animals were not examined microscopically, livers were normal. Urine volume of any of the NTA test groups did not differ from controls, but glycosuria was present in 5/7 of rats receiving 1% NTA. Mean fasting blood glucose levels were elevated in all groups receiving NTA comparing to control values. The NOAEL limited to the parameters of this test was 0.01% (approx. 10 mg/kg bw/day). The increased glucose levels at all doses were considered as treatment-related but not clearly adverse.

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

Ref.: 12

3.3.5.3. Chronic (> 12 months) toxicity

Oral

Rats

Male and females Sprague-Dawley rats were fed diets containing either 0, 0.03, 0.15 or 0.5% NTA (approx. 0, 19, 97, 322 mg/kg bw/day, purity 92.2%) or 0.5% of the calcium chelate of NTA (CaNaNTA, data not cited here) for up to 2 years. At 6, 12, 19 and 24 months, 5 animals of either sex were randomly selected for metabolic and histological studies of kidney lesions. Additional tissues taken for histologic examination after 19 and 24 months were spleen, liver, lung, heart, stomach, esophagus, small intestine, adrenal, trachea, urinary bladder, gonads, thyroid and bone (however no data were reported on the absence of evidence of abnormalities in these organs). The femurs were analysed for Zn and NTA content and other parameters (dry and ash weight, percent ash, fat phosphorus, copper, magnesium, calcium).

The feeding did not affect food consumption, feed efficiency, or growth of any test group during the first 8 weeks of the study (no further record thereafter). There was a dose-related trend in survival rates in dosed NTA-males, the survival rate was significantly lower in the high dose group. Liver body weight ratios were significantly higher than control values at 12 months for females receiving diets containing 0.5% NTA. A statistically significant increase in urinary zinc was intermittently noted in some rat groups receiving diets containing 0.15% and 0.5% NTA. In comparison to the control values, there was also a significant increase in the zinc level of bone in all test groups at nearly all check points during the experiment. The NTA deposition in the bone increased related to the dose, remaining constant during treatment and without any difference between sexes. The breaking strength of the bones from treated rats was not significantly altered from that of the control animals. No differences were noted microscopically in bones from test and control animals. In mid and high dose animals, lesions started at 6 months in 1 or 2 animals/group consisting of hydropic degeneration of the tubule and minor tubule dilatation.

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

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

Ref.: 13

Effects on the urinary tract

The organ system that was mainly affected by repeated oral treatment with NTA is the urinary tract with lesions at several sites: in the kidneys, ureters and urinary bladder. Histomorphologic kidney lesions were discovered at several segments: the cortex area, the renal papilla and the renal pelvis. The epithelium of the proximal convoluted tubules of the cortex region was found to be primarily affected. Another target tissue was the transitional cell epithelium (urothelium) of the renal pelvis, the ureters and the urinary bladder.

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

Ref.: 1

Comment

The EU RAR report on Trisodium Nitrilotriacetate (1) summarized the NOAELs and LOAELs from the oral repeated dose rat and mice studies. The estimated NOAELs varied from 10 mg/kg bw/day (10 week rat study) to 169 mg/kg bw/day (18 months + 3 months recovery mouse study). The estimated LOAELs varied from 97 mg/kg bw/day (2 year rat study) to 1400 (4 week rat study). It is proposed to use a NOAEL of 92 mg/kg bw/day for non-cancer endpoint. This NOAEL is derived from the 24 month cancer study (41). A NTA concentration applied as 2.5% NTA aqueous solution (2 ml, ≈ 50 mg/kg bw/d) was without irritation thus representing the NOAEL for local effects on the rabbit

dermis. SCCS concurs in a NOAEL of 92 mg/kg bw/day for non-cancer endpoint after oral administration.

3.3.6. Mutagenicity / Genotoxicity

Nitrilotriacetic acid and its sodium salts have been tested in a number of mutagenicity/ genotoxicity tests both *in vitro* and *in vivo*. The results will be summarized together below.

NTA was negative in the bacterial SOS DNA repair assay. NTA did not induce reverse mutation in *Escherichia coli* and *Salmonella typhimurium* or gene mutation in either *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. NTA induced micronuclei and chromosomal aberrations in plant cells, but it did not give rise to micronuclei in Chinese hamster lung cells *in vitro*. It weakly induced somatic mutation in *D. melanogaster*. It did not induce unscheduled DNA synthesis in rat primary hepatocytes in the absence of metabolic activation.

NTA did not induce gene mutation at the *hprt* locus of Chinese hamster lung V79 cells without exogenous metabolic activation or at the *tk* locus of mouse lymphoma L5178Y cells with or without exogenous metabolic activation. It did not induce sister chromatid exchange in Chinese hamster ovary cells or mouse lymphocytes *in vitro*. NTA induced chromosomal aberrations in rat-kangaroo kidney cells *in vitro* without exogenous metabolic activation and gene mutation in human cells *in vitro*. It did not induce sister chromatid exchange or chromosomal aberration in human lymphocytes *in vitro*.

NTA did not induce micronuclei or aneuploidy or dominant lethal mutation *in vivo* in mice with the exception of the acid except that the acid which induced aneuploidy in mouse germ cells.

It did not induce sex-linked recessive lethal mutation or dominant lethal mutation but induced aneuploidy in *Drosophila melanogaster*.

The results are summarized in Table 1.

Table 1: Genetic and related effects of nitrilotriacetic acid and its salts (Taken from IARC (1999, ref. 40))

Test system	**Results**[a]		**Dose**[b] (LED or HID)	**Reference**
	Without exogenous metabolic system	With exogenous metabolic system		
Nitrilotriacetic acid				
Escherichia coli WP2, reverse mutation	–	NT	4000	Zetterberg, 1970 (14)
Saccharomyces cerevisiae, forward mutation	–	NT	4000	Zetterberg, 1970 (14)
Saccharomyces cerevisiae, reverse mutation	–	NT	4000	Zetterberg, 1970 (14)
Schizosaccharomyces pombe, reverse mutation	–	NT	4000	Zetterberg, 1970 (14)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		1900 inj	Kramers , 1976 (15)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		4000	Woodruff et al., 1985 (16)
Drosophila melanogaster, dominant	–		1900 inj	Kramers, 1976

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
lethal mutations				(15)
Drosophila melanogaster, aneuploidy, germ cells	+		9600	Costa et al., 1988 (17)
Drosophila melanogaster, aneuploidy, germ cells	+		4000	Ramel & Magnusson, 1979 (18)
Sister chromatid exchange, Chinese hamster ovary cells in vitro	–	–	5	Loveday et al., 1989 (19)
Chromosomal aberrations, Chinese hamster ovary cells in vitro	–	–	5	Loveday et al., 1989 (19)
Dominant lethal mutation, mice in vivo	–		125	Epstein et al., 1972 (20)
Aneuploidy, mouse germ cells in vivo	+		275	Costa et al., 1988 (17)
Nitrilotriacetic acid, disodium salt				
Gene mutation, mouse lymphoma L5178Y cells, tk locus in vitro	–	NT	941	Toyokuni et al., 1995 (20)
Sister chromatid exchange, Chinese hamster lung V79 cells in vitro	–	NT	358	Hartwig et al., 1993 (21)
DNA damage (8-hydroxydeoxyguanosine formation), rat kidney *in vivo*	–		100 ip × 1	Umemura et al., 1990 (22)
Nitrilotriacetic acid, trisodium salt				
Echerichia coli PQ37, SOS chromotest	–	–	NR	Venier et al., 1987 (23)
Escherichia coli WP2, differential toxicity	+	+	250	Venier et al., 1987 (23)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	10 000 µg/plate	Dunkel et al., 1985 (24)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	870 µg/plate	Loprieno et al., 1985 (25)
Salmonella typhimurium TA100, reverse mutation	–	–	870 µg/plate	Venier et al., 1987 (23)
Escherichia coli WP2 uvrA, reverse mutation	–	–	10 000 µg/plate	Dunkel et al., 1985 (24)
Escherichia coli WP2 uvrA, reverse mutation (fluctuation test)	–	–	100 000	Venier et al., 1987 (23)
Saccharomyces cerevisiae, gene conversion	–	–	40	Loprieno et al., 1985 (25)
Aspergillus nidulans, genetic crossing-over	–	NT	10 930	Crebelli et al., 1986 (26)
Schizosaccharomyces pombe, forward mutation	–	–	40	Loprieno et al., 1985 (25)
Aspergillus nidulans, forward mutation	–	NT	18 510	Crebelli et al., 1986 (26)
Aspergillus nidulans, aneuploidy	–	NT	10 930	Crebelli et al., 1986 (26)
Allium cepa, micronuclei	+	NT	550	De Marco et al., 1986 (27)
Vicia faba, chromosomal aberrations	+	NT	1375	Kihlman & Sturelid, 1970 (28)
Drosophila melanogaster, somatic mutation (and recombination)	(+)		1336	Zordan et al., 1991 (29)
Micronucleus formation, Chinese	+	NT	514	Modesti et al.,

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
hamster lung Cl-1 cells in vitro				1995 (30)
Unscheduled DNA synthesis, rat primary hepatocytes in vitro	–	NT	1000	Williams et al., 1982 (31)
Gene mutation, Chinese hamster lung V79 cells, hprt locus in vitro	–	NT	1.5	Celotti et al., 1987 (32)
Gene mutation, mouse lymphoma L5178Y cells, tk locus in vitro	–	–	2350	Mitchell et al., 1988 (33)
Sister chromatid exchange, Chinese hamster ovary cells in vitro	–	NT	1.9	Loprieno et al., 1985 (25)
Sister chromatid exchange, Chinese hamster ovary cells in vitro	–	NT	1.0	Venier et al., 1985 (34)
Sister chromatid exchange, Chinese hamster ovary cells in vitro	–	NT	275	Ved Brat & Williams, 1984 (35)
Sister chromatid exchange, Chinese hamster ovary cells in vitro	–	NT	514	Montaldi et al., 1985 (36)
Sister chromatid exchange, mouse lymphocytes in vitro	–	NT	257	Montaldi et al., 1985 (36)
Chromosomal aberrations, rat kangaroo kidney PT K1 cells in vitro	+	NT	688	Kihlman & Sturelid, 1970 (28)
Gene mutation, human EUE cells DTR , in vitro	+	NT	3	Grilli & Capucci, 1985 (37)
Sister chromatid exchange, human lymphocytes in vitro	–	NT	275	Ved Brat & Williams, 1984 (35)
Chromosomal aberrations, human lymphocytes in vitro	–	NT	2063	Montaldi et al., 1988 (38)
Sister chromatid exchange, mouse bone-marrow cells in vivo	–		275 ip × 1	Russo et al., 1989 (39)
Aneuploidy, mouse bone-marrow cells in vivo	–		275 ip × 1	Russo et al., 1989 (39)
Micronucleus formation, mouse bone-marrow cells in vivo	–		400 ip × 1	Montaldi et al., 1988 (38)

a) +, positive; (+), weakly positive; –, negative; NT, not tested
b) LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, in-vitro test, µg/ml; in-vivo test, mg/kg bw/day; injection; i.p, intraperitoneal; NR, not reported

Comment
No data were available on the genotoxic and related effects of NTA in humans. NTA was mostly not genotoxic in mammalian cells *in vitro* or mutagenic to bacteria. NTA was not genotoxic in experimental systems *in vivo*, except that H_3NTA induced aneuploidy in mouse germ cells. NTA is not considered to be mutagenic / genotoxic.

3.3.7. Carcinogenicity

Mouse:

H_3NTA
Groups of 50 male and 50 female B6C3F1 mice, six weeks of age, were fed 7500 or 15 000 (maximum tolerated dose) ppm (752 and 1504 mg/kg bw/day) commercial-grade H_3NTA (purity 99.5%) in the diet for 18 months and were killed at 21 months. Groups of 20 male and 20 female mice served as controls. More weight loss was observed in high- and low-dose females and in high-dose males than in controls; survival was comparable in treated

and control animals of each sex. Hydronephrosis was detected in 8/44 (18%) high-dose males and 12/50 (24%) high-dose females, and animals of each sex had increased incidences of renal tumours, mostly adenocarcinomas: males — control, 0/20; low-dose (0%), 5/49 (10%); high-dose, 24/44 (55%) ($p < 0.001$), females — control, 0/20 (0%); low-dose, 0/39 (0%) ; high-dose, 4/50 (8%) ($p = 0.041$, test for linear trend).

Ref.: 41

$NTA.H_2O$
Groups of 50 male and 50 female B6C3F1 mice, six weeks of age, were fed 2500 or 5000 (maximum tolerated dose) ppm (169 and 338 mg/kg bw/day) commercial-grade NTA, trisodium salt, monohydrate (purity, 99.5%) in the diet for 18 months and were killed at 21 months. Groups of 20 male and 20 female mice served as controls. A dose-related decrease in body weight gain was observed in mice of each sex; survival was comparable in treated and control animals. No urinary-tract tumour was observed, but there was a dose-related increase in the incidence of haematopoietic tumours in male mice: control, 0/20; low-dose, 4/47; high-dose, 9/50 ($p = 0.015$, test for linear trend).

Ref.: 41

Na_2 $NTA.H_2O$
Groups of 40 male and 40 female random-bred Swiss mice, eight weeks of age, were given 5 g/l Na_2 $NTA.H_2O$ [purity unspecified] (167 mg/kg bw/day) in the drinking-water for 26 weeks and killed at 35-36 weeks. No increase in the incidence of tumours at any site was observed

Ref.: 42

Comment
The short duration of the experiment should be noted.

Rat:

H_3NTA
Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were fed 7500 or 15 000 (maximum tolerated dose) ppm (0, 526, and 1053 mg/kg bw/day) commercial-grade NTA (99.5% pure) in the diet for 18 months and were killed at 24 months. Groups of 20 males and 20 females served as controls. A modest, dose-related decrease in body weight gain was observed; survival was comparable in treated and control animals of each sex. Renal interstitial fibrosis and tubular dilatation were found frequently. Increases were observed in the incidences of urinary-tract tumours, mainly tubular-cell adenomas and carcinomas, in males: control, 0/20; low-dose, 1/49; high-dose, 7/48 ($p = 0.006$, test for linear trend); and of transitional and squamous-cell carcinomas of the urinary bladder in females: control, 0/18; low-dose, 2/45; high-dose, 12/48 ($p < 0.001$, test for linear trend). Increases were also seen in the incidences of phaeochromocytomas of the adrenal gland in females: control, 1/20; low-dose, 0/50; high-dose, 14/48 ($p < 0.001$). The incidences of liver tumours, all considered to be neoplastic nodules, were also increased in females: control, 2/15; low-dose, 8/49; high-dose, 22/49 ($p = 0.001$, test for linear trend)

Ref.: 41

$NTA.H_2O$
Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were fed diets containing 7500 or 15 000 (maximum tolerated dose) ppm (0, 355, and 724 mg/kg bw/day) commercial-grade $NTA.H_2O$ (purity, 99.5%) for 18 months and were killed at 24 months. Groups of 20 male and 20 female rats served as controls. A dose-related decrease in body weight was observed in rats of each sex; survival was comparable in treated and control animals. Evidence of renal inflammation was observed in low- and high-dose male and female rats, but no increase in the incidence of neoplasms was observed

Ref.: 41

Groups of 24 male and 24 female Fischer 344 rats, 51-55 days of age, were fed 0, 200, 2000 or 20 000 (maximum tolerated dose) ppm (0, 9, 92, 921 mg/kg bw/day) commercial-grade NTA.H_2O (no impurity detected) in the diet for 104 weeks. Decreases in body weight and survival and hydronephrosis were observed in high-dose males. One papilloma of the urinary bladder was seen in a female given 2000 ppm; all other kidney and urinary-tract tumours were observed in males and females given the highest dose. Tubular-cell adenomas and adenocarcinomas of the kidney were observed in 4/24 males [p = 0.004, Cochran-Armitage test]; transitional-cell carcinomas developed in the renal pelvis in 4/24 males (p = 0.003, Cochran-Armitage test) and in the ureter in 8/24 males (p < 0.001, Cochran-Armitage test); and metastases were observed in 5/24 males. Tubular-cell adenomas and adenocarcinomas of the kidney developed in 4/24 females (p = 0.003, test for linear trend); transitional-cell carcinomas developed in the ureter in 6/24 females (p < 0.001, test for linear trend) and in the urinary bladder in 5/24 females (p = 0.001, test for linear trend); metastases were observed in 5/24 females. A NOAEL of 92 mg/kg bw/day was derived from the study.

Ref.: 41

NTA
A total of 196 male, non-inbred Sprague-Dawley rats, weighing approximately 350 g, were given 0.1% NTA [purity unspecified] (100 mg/kg bw/day) in the drinking-water *ad libitum* for 704 days. A group of 192 untreated males served as controls. No difference in weight gain was observed between control and treated animals, but a higher proportion of treated rats died during the first 550 days of the study. An increase in the incidence of renal adenomas and adenocarcinomas was observed, with adenomas in 5/186 controls and 25 adenomas and 4 carcinomas in 29/183 treated animals (p < 0.01, Mantel-Haenszel test). In addition, renal tubular hyperplasia (grades III and IV) was observed 44/186 controls and 67/183 treated animals. There was no apparent difference in the frequency of severe nephritis between control and treated animals

Ref.: 43

Na_2 NTA.H_2O
Groups of 15 male and 15 female MRC rats, eight to ten weeks of age, were given approximately 20 ml of drinking-water containing 0.5% NTA, disodium salt [purity unspecified] (400 mg/kg bw/day) on five days a week for 84 weeks. All surviving animals were killed 104 weeks after the beginning of treatment. No significant difference in tumour incidence and no toxicity were observed

Ref.: 44

Comment
The small numbers of animals should be noted.

Initiation / promotion studies

<u>*Rat*</u>:

NTA.H_2O
In a two-stage carcinogenicity study of NTA.H_2O, four groups of 21 male Wistar rats, seven weeks old, received 0.05% *N*-nitroso(4-hydroxybutyl)butylamine (NHBBA) [purity unspecified] in the drinking-water for four weeks, after which they were fed 0 (NHBBA alone), 0.3%, 0.5% or 1.0% NTA.H_2O (purity, > 95%) in the diet for 28 weeks, when all survivors were killed. An increased incidence of papillary or nodular [transitional-cell] hyperplasia of the urinary bladder was observed with all three doses of NTA.H_2O: NHBBA alone, 3/20 (15%); low-dose, 13/21 (62%); mid-dose, 18/18 (100%); high-dose, 17/17 (100%) [p < 0.001, Cochran-Armitage test]. An increase was also detected in the incidence of [transitional-cell] papillomas of the urinary bladder: NHBBA alone, 0/20 (0%); low-dose,

1/21 (5%); mid-dose, 8/18 (44%); high-dose, 12/17 (71%) [$p < 0.001$, Cochran-Armitage test]. There was also an increased incidence of transitional-cell carcinomas of the urinary bladder: NHBBA alone, 0/20 (0%); low-dose, 1/21 (5%); mid-dose, 2/18 (11); high-dose, 7/17 (41%) [$p < 0.001$, Cochran-Armitage test]. In three additional groups that received 0.3%, 0.5% or 1.0% NTA.H_2O without NHBBA, simple [transitional-cell] hyperplasia of the urinary bladder was observed frequently

Ref.: 45

In a similar study, five groups of 25-26 male Fischer 344 rats were given 0, 0.01% or 0.05% NHBBA [purity unspecified] in the drinking-water for four weeks and were then fed diets containing 0 or 2% NTA.H_2O (95.0% pure) for 32 weeks, at which time survivors were killed. In animals treated with 0.05% NHBBA plus NTA.H_2O, an increased incidence of papillary or nodular [transitional-cell] hyperplasia of the urinary bladder was observed: NHBBA, 13/26 (50%); NHBBA plus NTA.H_2O, 23/26 (88%) ($p < 0.01$). [Transitional-cell] papillomas of the urinary bladder were also observed in 8/26 (31%) rats given NHBBA alone and in 18/26 (69%) also given the NTA compound ($p < 0.01$). In animals treated with the NTA compound alone, no hyperplastic or neoplastic lesion was observed

Ref.: 46

Comment
The short duration of the experiment should be noted.

Eight groups of 24 male inbred Wistar rats, seven weeks of age, were fed a diet containing 0 or 1000 ppm (mg/kg) *N*-nitrosoethylhydroxyethylamine (NEHEA) (purity, 99.8%) for two weeks after which they were given 0, 3000, 10 000 or 30 000 ppm NTA.H_2O (95.0% pure) in the diet for 30 weeks, at which time survivors were killed. A significant increase in the incidence of renal tubular-cell tumours was observed in animals treated with NEHEA plus the mid and high doses of the NTA.H_2O over that in animals treated with NEHEA alone: NEHEA alone, 4/24 (17%); low-dose, 5/22 (23%); mid-dose, 23/23 (100%) ($p < 0.01$); and high-dose, 23/23 (100%) ($p < 0.01$). In animals treated with the NTA compound alone, no renal tubular-cell tumour was observed

Ref.: 47

Comment
The short duration of the experiment should be noted.

Groups of 15-20 male Wistar rats, weighing 130-150 g, were given drinking-water containing 0.2% *N*-nitrosobis(2-hydroxypropyl)amine (NDHPA; purity, 98%) for two weeks and were then fed 1% H_3NTA (purity, 99%) or NTA.H_2O (purity, 95%) in the diet for 30 weeks. The tumour incidences in the groups treated with NDHPA, with NDHPA plus H_3NTA and with NDHPA plus NTA.H_2O were: urinary-bladder tumours (mainly papillomas) — 1/20 (5%), 1/20 (5%) and 7/20 (35%) [$p = 0.004$, Cochran-Armitage test]; renal cell tumours — 3/20 (15%), 15/20 (75%) and 10/20 (50%) [$p = 0.014$, Cochran-Armitage test]; and nephroblastomas — 3/20 (15%), 4/20 (20%) and 11/20 (55%) [$p = 0.003$, Cochran-Armitage test]

Ref.: 48

Comment
The short duration of the experiment should be noted.

General comment
Nitrilotriacetic acid and its sodium salt has been tested for carcinogenicity by oral administration in mice and rats. It induced renal tubular tumours (adenomas and

adenocarcinomas) in mice of each sex and haematopoietic tumours in male mice. It induced benign and malignant tumours of the urinary system (kidney, ureter and bladder) in rats of each sex. In addition, it induced hepatocellular andenomas in male and adrenal phaeochromocytomas in female rats. In two-stage studies of carcinogenicity in male rats treated by oral administration, nitrilotriacetic acid and its trisodium salt increased the incidence of urinary-tract tumours after pretreatment with various *N*-nitrosamines. IARC has concluded that there is *sufficient evidence* in experimental animals for carcinogenicity of nitrilotriacetic acid and its salts.

In relation to the mechanism of the carcinogenic effects of nitrilotriacetic acid and its salts, IARC (1999) concluded:

> "The nephrocarcinogenic effects of nitrilotriacetic acid in rats and mice appear to be related to dose-dependent changes in Zn^{++} homeostasis. Orally administered nitrilotriacetic acid and its trisodium salt were nephrotoxic to rats and mice of each sex. The toxicity occurs at high doses and appears to be due to Zn^{++} accumulation secondary to the chelating properties of nitrilotriacetic acid. Administration of zinc nitrilotriacetic acid or Zn^{++} accentuated the nephrotoxicity of nitrilotriacetic acid.
>
> Nitrilotriacetic acid has urothelial effects only in rats and at doses higher than those required for nephrotoxicity and proliferative effects. Although the mechanism of induction of the urothelial effects is not known, they are not related to Zn^{++} homeostasis but rather correlate with depletion of cellular calcium and possibly the formation of nitrilotriacetic acid-containing microcrystals.
>
> The renal and urothelial effects of nitrilotriacetic acid are associated with cellular toxicity and regenerative hyperplasia. Its toxic, regenerative proliferative and tumorigenic effects occur only at high doses. No direct genotoxic effect appears to be involved"

The EU Risk Assessment Report on Trisodium Nitrilotriacetate (ref. 1) concluded that the carcinogenic effects must be assumed to be relevant for humans.

The lowest dose with treatment-related increased tumour rates in the urinary tract of Sprague-Dawly rats with NTA was 100 mg/kg bw/day in a 2-year drinking water study (43). On the other hand, no significant difference in tumour incidence and no toxicity were observed in another smaller 2-year study with MRC rats receiving 400 mg/kg bw/day of $Na_2NTA.H_2O$ on 5 days a week for 84 weeks. One possible explanation for the difference could be the higher pH of NTA than of $Na_2NTA.H_2O$.

NTP (41) has carried out three rat studies with NTA after oral intake (added to the diet). In one study with H_3NTA (24 months) 1/49 low-dose (526 mg/kg/bw/day) and 7/48 high-dose male rats (1053 mg/kg bw/day) developed tubular-cell adenomas carcinomas. In a 18 month feeding study with $NTA.H_2O$ (355 and 724 mg/kg bw/day) the animals were killed at 24 months and no increase of neoplasms was observed. In a second 24 month study with $NTA.H_2O$ (9, 92, and 921 mg/kg bw/day), tubular-cell adenomas and adenocarcinomas in the kidney was only observed at the highest dose (921 mg/kg bw/day). Thus, in the NTP study the lowest dose giving a statistically significant increase in tumour frequency was 921 mg/kg bw/day. From the last study a NOAEL for carcinogenicity of rats is 92 mg/kg bw/day.

The lowest dose inducing urinary tumours in mice was 752 mg/kg bw/day of H_3NTA (41), while an increase of haematopoietic tumours in male mice was in another NTP study with $NTA.H_2O$ observed with 169 mg/kg bw/day.

A NOAEL for carcinogenicity of 92 mg/kg bw/day will be used in risk assessment.

3.3.8. Reproductive toxicity

Fertility impairment

Charles River CD) rats were exposed via diet for two successive generations. NTA (purity not given) was added to the diet of adult female rats in concentrations of 0.1% and 0.5% (90 and 450 mg/kg bw/day). Groups of 20 male and female rats each were fed these two dose levels continuously throughout two generations. The original F0 generation parental rats were bred three times: litters from the first breeding trial were followed up until weaning when they were discarded, descendants from the second breeding provided the F1 generation parental animals, litters from the third breeding were used for teratology studies. The second-generation (F1) animals were bred twice with the first litters followed up until weaning and the litters of the second breeding used for teratology studies.

Mean total food consumption (determined by week 8) at the high dietary level (0.5% Na_3NTA) was somewhat less than in the control and in the low dietary level (0.1% Na_3NTA) group in both sexes in the F0 generation and in the F1 males, statistically significantly different.

With respect to reproductive performance there were no significant differences in the conception rate during the specific phases of the study and no significant differences in the measurements of fertility and lactation in terms of average numbers of live-borns per litter, live pups on p.n. day 4, average number of weaned pups per culled litter and in the lactation index. Body weights of the new-borns were not reported. Offspring weaning weights were reduced at the 0.5% level in both sexes, an effect observed in litters of the first breeding trials and statistically significant for the F1 generation only, but not consistent across successive breedings and/or across generations.

Ref.: 49

Developmental toxicity

During the course of the above mentioned two-generation study in Charles River CD rats separate groups of animals derived from the third breeding trial of the F0 generation and from the second breeding trial of the F1 generation were used for additional teratological studies. Groups of 20 pregnant rats were exposed to diets containing 0.1% or 0.5% NTA during day 6 - 15 of gestation. Of each group ten dams were sacrificed on day 13 of gestation, respectively on day 21 of gestation. In dams sacrificed on day 13 of gestation the numbers of corpora lutea, implantations and resorptions were recorded. Foetuses removed from dams sacrificed on gestation day 21 were inspected for gross abnormalities, weighed and their sex determined and further investigated for skeletal and visceral defects.

Dams sacrificed on day 13 of gestation did not differ significantly from those sacrificed at day 21 of gestation in the number of corpora lutea, implantations or resorptions. Overall, the treatment groups did not differ significantly from control groups with respect to average number of corpora lutea, resorptions, live or dead foetuses or in the average weight of male and female foetuses.

Foetal evaluation for abnormalities did not reveal any skeletal defects. Soft tissue defects predominantly in the urinary system (hydroureter and /or hydronephrosis) were detected, however, the control groups of both generations were affected as much as any of the experimental groups. The authors therefore suggested that these effects were attributable to some unknown agent, possibly a virus.

Ref.: 49

During the course of the above studies, teratological studies were also performed on rabbits. Groups of 20 female New Zealand does were treated by gavage after artificial insemination with daily doses of 2.5, 25, 100, or 250 mg NTA in distilled water in a volume of 2 ml/kg bw during days 7 - 16 of gestation. One control group received water alone and another received no treatment at all. Animals were sacrificed on gestation day 28 and the offspring were treated in a way similar to that described for the rat study.

Data on the investigation of maternal parameters (food consumption, weight gain) were not given. As a result there were no significant differences in the average numbers of corpora lutea, resorptions, live and dead foetuses or in the average foetal body weights between treatment groups and controls. Also, no significant differences were seen in the number with gross abnormalities, skeletal or soft-tissue defects.

Ref.: 49

In a further study with mice the distribution and teratogenicity of nitrilotriacetic acid (NTA) was investigated Two groups of pregnant and nonpregnant NMRI or C57 Black mice were given 14C-labelled NTA intravenously, respectively per os, and the distribution of radioactivity at different time-intervals was studied with whole-body autoradiography. Two additional groups of ten NMRI mice each were either given 0.2% NTA via drinking water during g.d. 6 to 18 or taken as controls. Dams were weighed daily and at sacrifice on g.d. 18 evaluated for the numbers of resorptions and live foetuses. Foetuses were weighed and examined for external, skeletal and visceral defects.

The results from the autoradiographic study revealed a strong accumulation of radioactivity in the skeleton present up to 48 h, which was the longest time-interval studied. There was also a strong accumulation in the foetal skeleton thus indicating transplacental transfer.

During the course of application via drinking water, there were no significant differences in comparison to controls with respect to maternal weight gain during treatment, the numbers of resorptions, mean live foetuses (88 from controls/92 from treated) per litter or mean foetal weight. External, skeletal or visceral examination of the foetuses did not reveal any teratogenic effects.

Ref.: 50

Comments
The available studies in laboratory animals are not reported in sufficient detail. However, from available information of these studies it appears that NTA at dietary levels of approximately 450 mg/kg bw/day did not adversely affect reproductive performance and capability through two successive generations in rats. In addition, any specific teratogenic potential and/or impairment of embryo/foetal development was not indicated from the data for rats at 450 mg/kg bw/day and for rabbits at 250 mg/kg bw/day. It was demonstrated for mice that NTA probably passes the placenta and accumulates in the foetal skeleton.

3.3.9. Toxicokinetics

Conclusion from EU Risk Assessment (1).

"NTA, a metal ions chelating agent, is rapidly absorbed in dogs, rats and mice. The biological half-life for the elimination of NTA was approximately three hours. Studies in dogs, rats and mice indicated that NTA did not enter the enterohepatic circulation. The absorbed NTA was rapidly excreted via the urine except for a small fraction remaining in the bone. The urinary clearance is accomplished by passive glomerular filtration in rats. Any biotransformation of NTA was not observed in the studies for men, dogs, rats and mice. Less than one percent of the administered dose was excreted in the expired CO_2. Urinary calcium and zinc concentrations were increased in urine of rats and mice with high urinary NTA levels. The disposition of copper, iron and manganese was not appreciably influenced by oral NTA administration (up to 2% Na_2NTA or 1.5% H_3NTA in diet to rats). There are no

studies for skin permeation and no information is available on absorption via the inhalation route (aerosol, dust).

Highly variable urinary excretion of NTA has been reported for several species including humans: percentages of the dose excreted via urine were 12 (man), 14 (monkey), 23 (rabbit), 70 (rat), 80 (dog) and 96 (mouse). From experimental findings in bile cannulated animals (mice, rat, dogs) biliary excretion is less than 1%.

Based on the animal studies it may be appropriate to choose 50% as a default value for absorption of NTA after oral administration. However, human data point to a lower absorption rate. A value of 20% is proposed for risk characterisation purposes."

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

Discussed in the relevant sections.

3.3.12. Special investigations

3.3.12.1. Setting specific concentration limits

Guidance as to how potency considerations may be included in the setting of specific concentration limits for carcinogens which are classified according to the criteria of Directive 67/548/EEC is given in ref. 51.

As a general procedure, the potency calculations and subsequent assignment of specific concentration limits are only forwarded to the Working Group if they deviate from the general concentration limits. Potency is in this document defined as the magnitude, with respect to dose, of the carcinogenic activity of a chemical in the species under consideration. Carcinogens are subdivided into three potency groups of high, medium and low potency. For category 1a and 1b (previously carc. cat. 1 and 2), those of medium potency will normally be assigned to the general concentration limit of 0.1%. Carcinogens of high potency will normally be assigned a limit of 0.01% and carcinogens of low potency 1%. Category 2 carcinogens (previously carc. cat. 3) will normally be assigned to the medium potency group and given the general concentration limit of 1%, whereas highly potent Category 2 carcinogens will be assigned a specific concentration limit of 0.1% and Category 2 carcinogens of low potency a concentration limit of 1-5% on a case by case basis. The subdivision into the three potency groups is performed based on a tumorigenic dose descriptor. Among several possible descriptors, T25 is selected. T25, the dose giving a tumour incidence of 25% in an exposed human population, or in experimental animals after correction for the spontaneous incidence. Carcinogens of high potency are those with a T25 value which is: < 1 mg/kg bw/day, those of medium potency when: 1 mg/kg bw/day < T25 value < 100 mg/kg bw/day, and those of low potency when the T25 value is: > 100 mg/kg bw/day. In addition to subdividing carcinogens by the use of the tumorigenic dose descriptor, T25, several other elements bearing on tumorigenic potency (doseresponse relationships, site/species/strain/gender activity, mechanism including genotoxicity, mechanistic relevance to humans, toxicokinetics and other elements relevant to potency classification) are taken into consideration, which thereby may modify the potency preliminary evaluation.

Since the tumour induction with NTA is caused by a non-genotoxic mechanism and a threshold can be identified, the T25 is not suited for setting a specific concentration limit and a NOAEL should be used.

The NOAEL for carcinogenicity is 92 mg/kg bw/day. This would correspond to a T25 higher than 100 mg/kg bw/day if a T25 should be calculated.

A specific concentration limit of 5% was set for NTA in the Commission Regulation 790/2009.

Comment
SCCS notes that the specific concentration limits are based on potency considerations and not on risk assessment.

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Trisodium nitriloacetate (NTA)

The safety calculation is only considering dermal exposure

Exposure 17.8 g/day, 5% NTA (17.8 x 0.05x1000)		=	890 mg/day
Maximum absorption through the skin	890 x 0.1	=	89 mg/day
Typical body weight of human		=	60 kg
Systemic exposure dose (SED) bw/day	89 / 60	=	1.48 mg/kg

The NOAEL for carcinogenicity and non-cancer endpoints is 92 mg/kg bw/day. However, oral absorption in rat is considered to be 50%

	adjusted NOAEL	=	46 mg/kg bw/day

Margin of Safety	**NOAEL / SED** (46/1.48)	**=**	**31**

The MOS is too low to be accepted.

It should also be noted that the NOAEL for local effects on the intact or abraded skin was 2.5% NTA in 2 ml solution (50 mg/kg bw/day).

3.3.14. Discussion

The safety has only been evaluated for dermal exposure.

The stability of trisodium nitriloacetate in preparations is not reported.

Acute toxicity
NTA has low acute toxicity by oral, dermal, and inhalation routes.

Irritation /sensitization
Human data on local irritancy are not available. On the basis of the animal data currently available, proper assessment of skin and eye irritant/corrosive properties of the substance is not possible.

The EU Risk Assessment report (ref. 1) concluded that "Based on the limited data available and taking also into account the strongly basic pH-value of a 1% aqueous solution, the current classification of R36 - Irritating to eyes - is confirmed."

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

The negative data on patch tests with volunteers, published in 1971, cannot be used for evaluation since this study is based on a limited number of participants. No human case reports exist which point to a potential of NTA to cause skin sensitisation.

Dermal absorption
For 1% NTA dilutions, an absorption rate of 1% is taken for risk characterisation in humans based on an *in vitro* dermal absorption study. For higher NTA concentrations in solution and for animals, a default value of 10% absorption is taken for risk characterisation in the absence of adequate experimental data.

Repeated dose toxicity
The EU RAR report on Trisodium Nitrilotriacetate (ref. 1) has summarized the NOAELs and LOAELs from the oral repeated dose rat and mice studies. The estimated NOAELs varied from 10 mg/kg bw/day (10 week rat study) to 169 mg/kg bw/day (18 months + 3 months recovery mouse study). The estimated LOAELs varied from 97 mg/kg bw/day (2 year rat study) to 1400 (4 week rat study). It was proposed to use a NOAEL of 92 mg/kg bw/day for non-cancer endpoints. This NOAEL is derived from the 24 month NTP cancer study. The SCCS concurs with this proposal.

Mutagenicity/Genotoxicity
No data were available on the genetic and related effects of NTA in humans. NTA was mostly not genotoxic in mammalian cells *in vitro* or mutagenic to bacteria.NTA were not genotoxic in experimental systems *in vivo*, except that the acid induced aneuploidy in mouse germ cells. NTA is not considered to be mutagenic / genotoxic.

Carcinogenicity
Nitrilotriacetic acid and its sodium salt has been tested for carcinogenicity by oral administration in mice and rats. It induced renal tubular tumours (adenomas and adenocarcinomas) in mice of each sex and haematopoietic tumours in male mice. It induced benign and malignant tumours of the urinary system (kidney, ureter and bladder) in rats of each sex. In addition, it induced hepatocellular andenomas in male and adrenal phaeochromocytomas in female rats. In two-stage studies of carcinogenicity in male rats treated by oral administration, nitrilotriacetic acid and its trisodium salt increased the incidence of urinary-tract tumours after pretreatment with various *N*-nitrosamines. IARC has concluded that there is *sufficient evidence* in experimental animals for carcinogenicity of nitrilotriacetic acid and its salts.

In relation to the mechanism of the carcinogenic effects of nitrilotriacetic acid and its salts, IARC (1999) concluded that the nephrocarcinogenic effects of nitrilotriacetic acid in rats and mice appear to be related to dose-dependent changes in Zn^{++} homeostasis. The renal and urothelial effects of nitrilotriacetic acid are associated with cellular toxicity and regenerative hyperplasia. Its toxic, regenerative proliferative and tumorigenic effects occur only at high doses. No direct genotoxic effect appears to be involved. The EU Risk Assessment on Trisodium Nitrilotriacetate concluded with regard to carcinogenicity that the effect must be assumed to be relevant for humans.

A NOAEL for carcinogenicity of 92 mg/kg bw/day based on a NTP 2-year rat study will be used in risk assessment. NTA is considered to be a non-genotoxic carcinogen.

Reproduction toxicity
The available studies in laboratory animals are not reported in sufficient detail. However, from available information of these studies it appears that NTA at dietary levels of approximately 450 mg/kg bw/day did not adversely affect reproductive performance and capability through two successive generations in rats. In addition, no specific teratogenic potential and/or impairment of embryo/foetal development was indicated from the data for rats at 450 mg/kg bw/day and for rabbits at 250 mg/kg bw/day. It was demonstrated for mice that NTA probably passes the placenta and accumulates in the foetal skeleton.

Toxicokinetics and metabolism
NTA is rapidly absorbed in dogs, rats and mice. The biological half-life for the elimination of NTA was approximately three hours. Studies in dogs, rats and mice indicated that NTA did not enter the enterohepatic circulation. The absorbed NTA was rapidly excreted via the urine except for a small fraction remaining in the bone. The urinary clearance is accomplished by passive glomerular filtration in rats. No biotransformation of NTA was observed in the studies for men, dogs, rats and mice. Urinary calcium and zinc concentrations were increased in urine of rats and mice with high urinary NTA levels. The disposition of copper, iron and manganese was not appreciably influenced by oral NTA administration. No information is available on absorption via the inhalation route (aerosol, dust).

Highly variable urinary excretion of NTA has been reported for several species including humans: percentages of the dose excreted via urine were 12 (man), 14 (monkey), 23 (rabbit), 70 (rat), 80 (dog) and 96 (mouse). From experimental findings in bile cannulated animals (mice, rat, dogs) biliary excretion is less than 1%.

The EU Risk Assessment report (1) concluded that "Based on the animal studies it may be appropriate to choose 50 % as a default value for absorption of NTA after oral administration. However, human data point to a lower absorption rate. A value of 20% is proposed for risk characterisation purposes." ... "a default value of 20% is proposed for uptake via inhalation."

4. CONCLUSION

Based on a worst case assessment with a maximum use concentration of 5% NTA in cosmetic products and a dermal absorption of 10%, the Margin of Safety is considered to be too low. There is an absence of specific information on the actual concentrations of NTA present in cosmetic products and specific measurement of dermal absorption of it through skin at appropriate concentrations. Information of the irritant potential on skin at maximum use concentrations is lacking.

With the information available at the time of assessment, the SCCS is of the opinion that the presence of NTA with a maximum use concentration of 5% in cosmetic products is not safe for the consumer. A re-evaluation may be possible should relevant data that addresses the above be provided.

5. MINORITY OPINION

Not applicable

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