EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

Brussels, C7/GF/csteeop/edta-hh/100903 D(03)

SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE)

Opinion on the results of the Risk Assessment of:

TETRASODIUM ETHYLENEDIAMINE TETRAACETATE (NA4EDTA)
CAS N°: 64-02-8

and

EDETIC ACID (EDTA)
CAS NO. 60-00-4

HUMAN HEALTH PART

Carried out in the framework of Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances[1]

Adopted by the CSTEE during the 39th plenary meeting of 10 September 2003

[1] Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of those substances if they are produced or imported into the Community in volumes above 10 tonnes per year. The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC)1488/94, which is supported by a technical guidance document.

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INTRODUCTION

EDTA is used both as the free acid and as the sodium salt. Both compounds are applied for a variety of purposes and present in a large number of formulations.

Terms of Reference

In the context of Regulation 793/93 (Existing Substances Regulation) and on the basis of the examination of the Risk Assessment Report provided by the European Chemicals Bureau, the CSTEE is invited to examine the following issues:

(1) Does the CSTEE agree with the conclusions of the Risk Assessment Report?

(2) If the CSTEE disagrees with such conclusions, the CSTEE is invited to elaborate on the reasons for this divergence of opinion.

GENERAL COMMENTS

The document follows the recommendations of the TGD and is comprehensive and well written. Regarding human health related endpoints, the documents on the free acid and the sodium salt are almost identical and cross reading of data from different salts of EDTA and the free acid has been performed for hazard assessment except for the endpoints acute toxicity, irritation, corrosivity and sensitisation. The cross reading is justified by the dissociation of both compounds in biological media. The CSTEE supports the cross reading as appropriate and therefore also provides only one comment on the two RARs.

SPECIFIC COMMENTS

1. Exposure assessment

Edetic acid. For occupational exposure to edetic acid, the inhalation and dermal routes are considered as important pathways. Regarding consumer exposure, dermal and oral exposures are characterised.

To characterise worker exposure by inhalation and dermal contact, three scenarios are developed in the RAR. These are production and further processing of edetic acid as chemical intermediate, formulation of solutions and powdery products containing edetic acid, and the use of these formulations. Since measured workplace air concentrations are not available, typical work place air concentrations are estimated using EASE. Regarding dermal exposure, due to expected low uptake by the dermal route, no quantitative conclusions are made. Doses received by inhalation are based on the assumption of 100 % absorption of the inhaled material. This has to be considered a worst case scenario due to the large average particle size of edetic acid powders.

Consumer exposure to edetic acid may occur from cosmetics and from residues in food contact material, but consumer exposure is predicted to be low. Considering the low extent of dermal absorption, only very low systemic doses are predicted from dermal exposure.

Na_4EDTA. To characterise worker exposure to Na_4EDTA, identical scenarios as developed for edetic acid are used and it is concluded that exposure will mainly occur by inhalation of dust generated when handling powdered Na_4EDTA. Exposure estimates are also mainly based on calculations using EASE; due to the very low penetration of the Na_4EDTA through the skin, the dermal exposure route is not considered to be relevant.

2. Effects assessment

As mentioned before, most of the health effects are assessed by cross reading and therefore this chapter is highly overlapping for the two compounds.

Acute toxicity

In experimental animals, both edetic acid and the Na_4EDTA show only low potential for toxicity and LD_{50} values are in the range of 2 g/kg and above.

Irritation, corrosivity and sensitisation

Both edetic acid and Na_4EDTA are mild skin irritants, but comparatively potent eye irritants. Based on limited experimental data and lack of reports of skin and respiratory sensitisation during industrial use of edetic acid and Na_4EDTA, the rapporteur concludes that both edetic acid and Na_4EDTA do not cause sensitisation by skin contact or by inhalation. Based on the positive results with the Magnusson-Klingman test, the CSTEE concludes that edetic acid salts may be weak skin sensitisers. Since there are some indications from human studies on skin sensitisation, this aspect should be investigated further. In line with risk assessment report, the CSTEE concludes that the bronchoconstriction following inhalation exposure may be related to calcium ion chelation, a non-immunological mechanism..

Toxicokinetics

After oral administration of edetic acid, gastrointestinal absorption is poor and accounts for < 20 % of dose. Absorbed material is rapidly excreted with urine and only negligible amounts are metabolised to CO_2. Absorption after inhalation has not been studied.

Repeated dose toxicity

Based on a two-year study after dietary exposure, a NOAEL of 500 mg/kg/day is derived for Na_3EDTA. Based on this study and a limited number of further non-standard repeated dose toxicity studies, it is concluded that both edetic acid and Na_4EDTA have only a low potential for toxicity after repeated oral administration.

The CSTEE supports this conclusion and the derived NOAEL.

Genotoxicity

The mutagenicity and genotoxicity of edetic acid and its salts was studied in several in vivo and in vitro assays. No genotoxicity tests with Na_4EDTA are reported, and all data presented refer either to the free acid (EDTA) or trisodium EDTA trihydrate (Na_3EDTA x 3 H_2O; CAS No. 150-38-9).

In vitro

No bacterial mutagenicity tests were available for EDTA. Trisodium EDTA trihydrate (Na_3EDTA x 3 H_2O; CAS No. 150-38-9) was tested negative in the Ames test with and without metabolic activation.

In mammalian cells, EDTA induced 2- to 6-fold increases in mutant frequencies in the mouse lymphoma forward mutation assay at high, cytotoxic concentrations (25 and 30mM), which decreased pH from 7.2 to 6.1 and 5.8, respectively. It should be noted in the RAR that the concentrations applied were clearly in excess of the recommended top concentrations for *in vitro* tests (10 mM).

Whilst DNA single strand breaks were induced with EDTA in mouse lymphoma cells at, again, extremely high concentrations (≥ 40mM), no such effects were found in V79 cells. As the toxicological profile of EDTA is determined by the formation of chelates with metal ions, the genotoxicity tests with extremely high EDTA concentrations in culture medium should be treated with caution, as the effects may be indirect, resulting from the loss of bioavailability of essential elements. The RAR should comment on this issue more extensively.

In addition to the Ames tests with Na_3EDTA x 3 H_2O already presented in the RAR, further genetic toxicology information on this chemical is available from the NTP database, and should be included in the RAR. The data include negative results in a chromosome aberrations test, in a sister chromatid exchange test, and in the mouse lymphoma test (NTP, 2003).

In vivo

No *in vivo* studies were reported for EDTA.

In studies performed in accordance with current guidelines, Na_2EDTA was not clastogenic in polychromatic erythrocytes and bone marrow cells of male mice after oral and intraperitoneal administration, respectively (500-2000 mg/kg bw, 2x p.o.; 186 mg/kg bw i.p.). In a less reliable study, a dose-dependent increase in the incidence of micronucleated polychromatic erythrocytes

was reported after oral administration of Na_2EDTA at relatively low doses (15 and 20 mg/kg bw) to mice. Aneuploidy and sister chromatid exchanges were not observed in bone marrow cells of mice after a single i.p. administration of 93 and 186 mg/kg bw.

In germ cells of mice, Na_2EDTA (93 or 186 mg/kg bw i.p.) did not induce chromosomal aberrations in spermatogonia and did not cause aneuploidy in primary and secondary spermatocytes. A dominant lethal test was also negative (10 mg/kg bw p.o. for 5 consecutive days). A positive result was obtained in a micronucleus test with spermatids, indicating that aneugenic effects may be induced in late spermacytogenesis. The effect was noted at a very high dose level (186 mg/kg bw i.p.) near to the LD_{50} value, and was therefore considered to be of no practical relevance (a threshold below which no toxicity would occur is commonly accepted for this type of effect). In *Drosophila*, Na_2EDTA was negative in the somatic mutation and recombination test (SMART), but induced aneuploidy in germ cells. EDTA caused chromosomal loss in Drosophila germ cells.

In summary, genotoxic effects were seen at extremely high dose levels, probably involving secondary mechanisms. The RAR concludes that edetic acid and Na_4EDTA are not expected to be mutagenic in humans based on the assumption of a threshold mode of action for the induction of aneuploidy.

Carcinogenicity

A carcinogenicity study according to current guidelines was performed with Na_3EDTA. The results from these feeding studies conducted on trisodium EDTA trihydrate ($Na_3EDTA \times 3\ H_2O$; CAS No. 150-38-9) by the U.S. National Cancer Institute in 1977 are presented in detail in the RAR. The test material was administered to Fischer 344 rats and B6C3F1 mice for 103 weeks (3750 or 7500 ppm, corresponding to 248 and 495 mg/kg bw/day in rats, and 469 and 938 mg/kg bw/day in mice, respectively). No compound-related signs of clinical toxicity were noted, and, although a variety of tumours occurred among test and control animals of both species, no tumours were related to treatment with the test material.

Based on these results, it was concluded that there is no concern for EDTA with regard to carcinogenicity. Although the CSTEE, in principle, agrees with this conclusion, it recommends that further justification and additional information is provided in the RAR. This should include a statement with regard to genotoxicity, the results of the cell transformation assays with EDTA (all negative, *cf.* Fukuda et al., 1987; LeBoef et al., 1996; Matthews et al., 1993; Tsutsui et al., 1987), and a justification for using data from $Na_3EDTA \times 3\ H_2O$.

It should be mentioned in the RAR that epidemiological studies were not available for evaluation available.

The CSTEE supports this conclusion.

Reproductive and developmental toxicity

A number of studies on effects of edetic acid and some salts after oral administration are available. A teratogenic effect of edetic acid salts has been observed after doses above 1000 mg/kg/day. The RAR discusses possible modes of action and concludes that teratogenicity is most likely due to zinc depletion by the very high doses applied, but does not derive an oral NOAEL. The CSTEE accepts the conclusion that teratogenicity of edetic acid salts is a high dose effect.

3. Risk characterisation

Acute and repeated-dose toxicity

Regarding worker exposure, the MOS values for inhalation toxicity for both edetic acid and Na_4EDTA are mostly well above 100 and therefore conclusion ii) is reached and supported by CSTEE. Exposure scenario 1 gives MOS values between 30 and 70 regarding inhalation toxicity. The RAR also derives conclusion ii) due to a conservative exposure assessment. The CSTEE supports this conclusion since the calculated received doses are also be considered as worst case scenario due to the comparatively large particle size of the powdery material, which will not penetrate into the lung, and therefore results in < 100 % absorption. The RAR defines minimal acceptable MOS values based on a number of adjustment factors whose validity may be questioned. It is proposed that the RAR should just justify why conclusion ii) for scenario 1 was reached.

Genotoxicity and Carcinogenicity

No mutations were induced in bacteria. An increase in mutant frequency and DNA damage were found in cultures of mammalian cells after exposure to high concentrations of the free acid which exceeded current recommendations for *in vitro* tests. *In vivo*, there was no indication of a clastogenic activity in somatic cells. At doses near the LD_{50} value, aneugenic effects were found in germ cells of mice.

The CSTEE agrees with the rapporteur that EDTA shows genotoxic activity at extremely high dose levels, most probably involving secondary mechanisms.

Na_4EDTA has not been tested for its carcinogenic properties nor were epidemiological data available. There is, however, no evidence of carcinogenicity from lifetime studies conducted on $Na_3EDTA \times 3\ H_2O$ in rats and mice.

Based on the available genotoxicity data for EDTA and its salts, the negative data from cell transformation assays and based on negative carcinogenicity data from studies with $Na_3EDTA \times 3\ H_2O$, the CSTEE agrees with the member states' rapporteur that there are no evident concerns regarding this endpoint.

The CSTEE therefore supports conclusion (ii) for workers and consumers.

Reproductive and developmental toxicity

Conclusion ii) is reached regarding effects on development and fertility for workers and consumers despite a MOS of < 100 for scenario 1 regarding workplace exposure. The RAR again justifies reaching conclusion ii) by deriving a minimal acceptable MOS. Due to the conservative exposure assessment, the steep dose-response, and a plausible mechanism to explain teratogenicity of edetic acid salts (zinc depletion) the CSTEE supports this conclusion.

Skin sensitisation.

Regarding this endpoint, CSTEE does not agree with conclusion ii) and supports conclusion i).

REFERENCES

CCRIS (1995). Sodium Edetate. CAS-Nr. 64-02-8. Short-Term Test Program Sponsored by the Division of Cancer Aetiology, National Cancer Institute, Dr. David Longfellow, Project officer, p. Y95. CCRIS Record Number 6797. Available from the TOXNET database.

Fukuda, S. (1987). In: Isfort et al, *Mutat. Res*., *356, 11-63*, 1996.

LeBoeuf, R.A., Kerckaert, G.A., Aardema, M.J., Gibson, D.P., Brauniger, R. & Isfort, R.J. (1996). The pH 6.7 Syrian hamster embryo cell transformation assay for assessing the carcinogenic potential of chemicals. *Mutat. Res. 356,85-127.*

Matthews, E.J., Spalding, J.W. & Tennant, R.W. (1993). Transformation of BALB/c 3T3 cells: V. Transformation responses of 168 chemicals compared with mutagenicity in Salmonella and carcinogenicity in rodent bioassays. *Environ. Health Perspectives*, *101 (Suppl. 2), 347–482.*

NTP (2003). NTP Study Overviews and General Protocols. Testing Status: Trisodium Ethylenediaminetetraacetate Trihydrate (EDTA). Last update 08/11/2003. *http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatt/C03974.html*

Tsutsui, T., Suzuki, N., Kobayashi, Y., Suzuki, H., Fukuda, S. & Maizumi, H. (1987). Assessment of the Carcinogenic Hazard of 27 Substances used in Dental Practices. 60th General Meeting of the Japanese Pharmacological Society, Chiba, Japan, March 29 – April 1, 1987. Jpn *J Pharmacol 43 (Suppl). 132 p.*