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Edetic acid (EDTA) in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

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Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as International Standards for Drinking-Water. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO Guidelines for drinking-water quality (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared/updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants examined in drinking-water.

For each chemical contaminant or substance considered, a lead institution prepared a health criteria document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the requested health criteria documents.

Under the responsibility of the coordinators for a group of chemicals considered in the guidelines, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the experts meetings. A "final task force" meeting reviewed the health risk assessments and public and peer review comments and, where appropriate, decided upon guideline values. During preparation of the third edition of the GDWQ, it was decided to include a public review via the world wide web in the process of development of the health criteria documents.

During the preparation of health criteria documents and at experts meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health

Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the joint FAO/WHO Meetings on Pesticide Residues, and the joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO internet site and in the current edition of the GDWQ.

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The work of the following coordinators was crucial in the development of this document and others in the Addendum:

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GENERAL DESCRIPTION

Identity

CAS no.: 60-00-4
Molecular formula: $C_{10}H_{16}N_2O_8$
Edetic acid (ethylenediaminetetraacetic acid) and its salts are commonly referred to as EDTA. Other names include *N*,*N*'-1,2-ethanediylbis[*N*-(carboxymethyl)glycine], Versene acid, and (ethylenedinitrilo)tetraacetic acid.

Physicochemical properties

Property	*Value*
Physical appearance	Colourless crystals
Solubility in water	0.5 g/litre at 25°C

Organoleptic properties

EDTA has a slightly salty taste.

Major uses

EDTA has been used extensively in medicine as a chelating agent for the removal of toxic heavy metals. The disodium salt of EDTA is a common component in many eye drops and contact lens wetting and cleansing solutions. EDTA is also used in a number of personal care and hygiene products, such as shampoos, liquid soaps, creams, and lotions.

Household disinfectants often contain EDTA, especially if fatty acid soaps are used in the disinfectant formulation. These soaps are sensitive to calcium and magnesium, and the chelating agent prevents the formation of hard-water soap curds (Hart, 1984).

EDTA is also used as a food additive in a range of products, including canned shrimp and prawns, canned mushrooms, and frozen french fries. It is added to salad dressings to prevent rancidity.

EDTA is used in many industrial processes, in agriculture, in photochemicals, pharmaceuticals, and textiles, and in galvanizing and paper manufacturing. The usage of EDTA in West Germany in 1986 by industry was: metal processing and galvanizing technology, 30%; detergents, 20%; photographic industry, 20%; textiles, 10%; paper, 5%; and miscellaneous (antioxidants in soaps and cosmetics, pharmaceuticals, and foodstuffs), 15%. The total use over the year was about 15 000 t (Brauch & Schullerer, 1987).

Environmental fate

Once EDTA is present in the aquatic environment, its speciation will depend on the water quality and the presence of trace metals with which it can combine. The fate and behaviour of the different complexes may vary considerably. The iron(III) EDTA complex is the most labile because it is very photo-active. Svenson et al. (1989) calculated a half-life of 11 minutes for the photolysis of iron(III) EDTA dissolved in water and irradiated with sunshine equivalent to the annual maximum intensity. The photo-oxidation of free EDTA in water at pH 9.0 has a measured half-life of 36 years for an initial hydroxyl radical concentration of 5×10^{-9} mol/litre and a 12-hour daylight duration. The iron(III) EDTA will exchange slowly with other trace metals after discharge to the environment, a process that will be dependent upon the pH of the water, as each trace metal has an optimum pH for chelation. Other metal complexes of EDTA are much more persistent and are not readily biodegraded in the aquatic

environment. In soil–water systems, degradation has been observed, but the extent varies with soil type and length of exposure. The removal of EDTA from communal wastewater by biodegradation in sewage purification plants is very limited. A limited removal takes place by adsorption on sludge. Similarly, elimination of EDTA by different treatment methods of drinking-water is negligible, including filtration on activated carbon. The most effective elimination is by ozonation (Gilbert & Beyerle, 1992).

There has been concern that EDTA mobilizes heavy metals in the environment. However, based on stoichiometry, 40 µg of EDTA per litre (the maximum concentration observed in the Rhine and Meuse rivers) would complex 4–15 µg of metals per litre at most, and this would be likely to pose problems for drinking-water only with regard to cadmium. A further modifying factor is that the effect on cadmium leaching will be limited because the EDTA is primarily bound to other metals at these concentrations (van Dijk-Looyard et al., 1990). For the majority of fresh waters, EDTA will be associated largely with calcium, provided that the EDTA is not present in stoichiometric excess. For waters of pH lower than 6.0, however, competition from hydrogen ions for available ligand assumes greater importance.

ANALYTICAL METHODS

EDTA can be analysed by potentiometric stripping analysis (Fayyad et al., 1988). This method, which has been used to detect EDTA in a wide variety of wastewater and natural water samples, has a detection limit of 1 µg/litre.

ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Water

Most of EDTA's uses will result in its release to the aquatic environment. It has been estimated that concentrations of 50–500 µg/litre are present in wastewaters. Annual average concentrations of EDTA in European surface waters ranged between >1 and >60 µg/litre, and a concentration of 900 µg/litre was found in the Zerka River in Jordan (van Dijk-Looyard et al., 1990). Measured concentrations in natural waters were also reported to range from 10 to 70 µg/litre, with a median value of 23 µg/litre (Frank & Rau, 1990). Mean EDTA concentrations at 45 different sampling points on 29 different rivers of Germany in 1993 ranged between almost 50 µg/litre (Lippe River at Wesel) and a few µg/litre, with most annual mean values being between 5 and 15 µg/litre (EFA-Germany, 1995). EDTA has also been detected in surface waters and in drinking-water prepared from surface waters at concentrations of 10–30 µg/litre (van Dijk-Looyard et al., 1990).

Food

EDTA's use as a food additive has been limited. The maximum levels of EDTA in canned shrimp and prawns, canned mushrooms, and frozen french fries are 250, 200, and 100 mg/kg, respectively (Smith, 1990).

Estimated total exposure and relative contribution of drinking-water

Human exposure to EDTA arises directly from its use in food additives, disinfectants, medicines, and personal care and hygiene products. Exposure to EDTA from drinking-water is probably very small in comparison to exposure from other sources.

KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Metabolism in laboratory animals and humans

Foreman et al. (1953) examined the metabolism of ^{14}C-labelled calcium disodium EDTA in the rat in two series of experiments involving the administration of doses of 50 mg/kg of body weight, intraperitoneally, intravenously, intramuscularly, or orally by intubation, to Sprague-Dawley rats. The studies indicated that calcium disodium EDTA was poorly absorbed from the gastrointestinal tract. About 80–95% of the dose appeared in the faeces after 24 hours. The amount absorbed in 24 hours, determined from the quantity found in the tissues and urine, ranged from 2 to 18%, with most of the values being between 2 and 4%. At the low pH of the stomach, the calcium chelate is dissociated with subsequent precipitation of the free acid, and this is only slowly redissolved as it passes through the alimentary tract. Increasing the dose is not necessarily a good way of increasing the amount absorbed, as the rats exhibited diarrhoea at higher doses. Srbrova & Teisinger (1957) confirmed the dissociation of the calcium chelate in the stomach. When a dose of 200 mg of calcium disodium EDTA was introduced into the duodenum of rats, the authors found absorption to be 6.5–26%.

Experiments in humans also revealed poor absorption; only 2.5% of a 3-g dose of calcium disodium EDTA was excreted in the urine (Srbrova & Teisinger, 1957). Only 5% of a dose of 1.5 mg of ^{14}C-labelled calcium disodium EDTA given in a gelatin capsule to normal healthy men was absorbed (Foreman & Trujillo, 1954). EDTA has also been shown to be rapidly excreted from the body. Intravenous doses of 3 g of radiolabelled calcium disodium EDTA, given to two subjects, were almost entirely excreted within 12–16 hours (Srbrova & Teisinger, 1957).

A summary of a 1956 Ph.D. thesis by Chan in Anonymous (1964) reported biochemical studies with disodium EDTA. In a study in rats, 32 hours following administration of a single oral dose of 95 mg of disodium EDTA per rat, 93% of the dose was recovered from the colon. After doses of 47.5, 95, and 142.5 mg of disodium EDTA, the amount of EDTA recovered in the urine was directly proportional to the dose given, suggesting that EDTA was absorbed from the gastrointestinal tract by passive diffusion.

Metal complexation with EDTA

EDTA is a hexadentate chelator capable of combining stoichiometrically with virtually every metal in the periodic table (Chaberck & Martell, 1959). With divalent or trivalent metal ions, a neutral or anionic metal chelate results. The metal is largely prevented from reacting with competing anions, and its solubility is greatly increased. The effectiveness of EDTA as a chelate for a particular metal ion is given by its stability constant with the metal ion. Chelation potential is affected by pH, the molar ratio of chelate to metal ion, and the presence of competing metal ions capable of forming complexes with EDTA (Plumb et al., 1950; Martell, 1960; Hart, 1984). The stability constants for different metal–EDTA complexes vary considerably, and any metal that is capable of forming a strong complex with EDTA will at least partially displace another metal.

Of the nutritionally important metals, Fe^{3+} has the highest stability constant ($\log k = 25.1$), followed by Cu^{2+} with 18.4, Zn^{2+} with 16.1, Fe^{2+} with 14.6, Ca^{2+} with 10.6, Mg^{2+} with 8.7, and Na^{+} with 1.7 (West & Sykes, 1960). The situation is somewhat complicated by each metal having an optimum pH for chelate formation, ranging from pH 1 for Fe^{3+} to pH 3 for Cu^{2+}, pH 4 for Zn^{2+}, pH 5 for Fe^{2+}, pH 7.5 for Ca^{2+}, and pH 10 for Mg^{2+} (West & Sykes, 1960). When sodium iron EDTA is ingested with foods, the Fe^{3+} ion would be expected to remain firmly bound to the EDTA moiety during passage through the gastric juice, but it could be exchanged for Cu^{2+}, Zn^{2+}, Fe^{2+}, or Ca^{2+} in the duodenum (WHO, 1993).

In biological systems, Ca^{2+} will usually be most accessible to EDTA. In general, zinc seems to be the next most accessible. About 80% of the zinc in liver is freely available to EDTA. The overall availability of the other physiologically important metals is probably in the order copper > iron > manganese > cobalt (Chenoweth, 1961). EDTA removes about 1.4% of the total iron from ferritin at pH 7.4 to form an iron chelate (Westerfield, 1961).

Perry & Perry (1959) investigated the changes in normal concentrations of trace metals in human urine following administration of EDTA. Calcium disodium EDTA had been observed to lower the level of cholesterol in human plasma, and therefore this study investigated metal concentrations in consecutive 24-hour urine samples from hypercholesterolaemic patients before, during, and after the intravenous administration of calcium disodium EDTA. The results indicated a 10-fold increase in urinary excretion of zinc during the administration of calcium disodium EDTA. A smaller effect on cadmium and manganese may have occurred, but the results were not clear, because some of the control concentrations were too low to quantify. There were also suggestive increases in the excretion of lead and vanadium. Foreman (1961) reported that EDTA enhanced the excretion of cobalt, mercury, manganese, nickel, lead, thallium, and tungsten.

When EDTA is present in food, iron (primarily Fe^{3+}) remains complexed with EDTA under the acidic conditions prevailing in the stomach. The chelate holds the iron in solution as the pH rises in the upper small intestine, but the strength of the complex is progressively reduced, allowing at least partial exchange with other metals and the release of some of the iron for absorption. There is convincing evidence that iron chelated by EDTA (sodium iron EDTA) is available for absorption via the physiologically regulated pathways responsible for iron uptake (Candela et al., 1984). The results of the absorption studies with sodium iron EDTA indicate that iron is dissociated from the EDTA moiety prior to absorption.

Hurrell et al. (1994) examined the influence of sodium iron(III) EDTA, used as a food fortificant, on the metabolism of calcium, zinc, and copper in the rat. The results showed that changing the iron fortificant from iron sulfate to sodium iron(III) EDTA increased the apparent zinc absorption, retention, and excretion in the rats receiving the zinc-deficient diets. The authors suggest that the study appears to indicate a beneficial effect on zinc nutritional status following addition of sodium iron(III) EDTA as an iron food fortificant. However, the results are complicated by the fact that iron status is an important factor in zinc metabolism and a high level of iron inhibits the availability of zinc. In addition, the diets contained soybean protein, which is high in phytate, an inhibitor of both iron and zinc absorption. These factors make it difficult to draw any definite conclusions from this study.

EFFECTS ON EXPERIMENTAL ANIMALS AND *IN VITRO* TEST SYSTEMS

Acute exposure

Acute toxicity studies have been carried out with disodium EDTA and calcium disodium EDTA in laboratory animals. LD_{50} values (mg/kg of body weight) reported in these studies are summarized below:

Rat	Oral: 2000–2200, Na_2EDTA	(Yang, 1952)
	Oral: 10 000 ± 740, $CaNa_2EDTA$	(Oser et al., 1963)
Rabbit	Oral: 2300, Na_2EDTA	(Shibata, 1956)
	Oral: ~7000, $CaNa_2EDTA$	(Oser et al., 1963)
	Intraperitoneal: ~500, $CaNa_2EDTA$	(Bauer et al., 1952)
Dog	Oral: ~12 000, $CaNa_2EDTA$	(Oser et al., 1963)

Oser et al. (1963) reported that the oral LD_{50} in rats was not affected by the presence of food in the stomach or by pre-existing deficiency in calcium, iron, copper, or manganese.

Short-term exposure

In a study involving the intraperitoneal administration of 250 or 500 mg of calcium disodium EDTA per kg of body weight per day to groups of five male rats for 3–21 days, it was reported that weight gain was satisfactory and that the histology of the lung, thymus, liver, spleen, adrenal, small gut, and heart was normal; there was a mild to moderate effect on the kidney (Reuber & Schmieller, 1962).

Groups of five male rats were given 250, 400, or 500 mg of disodium EDTA per kg of body weight per day intraperitoneally for 3–31 days; some groups were observed for an additional 2 weeks. At 500 mg/kg of body weight per day, all rats became lethargic and died within 9 days; the kidneys were pale and swollen, and there was moderate dilation of the bowel and subserosal haemorrhages. Histological examination of a number of organs showed lesions only in the kidneys. Animals at the 400 mg/kg of body weight per day dose level died within 14 days; kidney and bowel symptoms were similar to those seen at the high dose. One rat in the 250 mg/kg of body weight per day dose group showed haemorrhage of the thymus. All three groups exhibited varying degrees of damage to the kidney, with recovery occurring on withdrawal of the disodium EDTA (Reuber & Schmieller, 1962).

Four groups of one male and three female mongrel dogs were fed diets containing 0, 50, 100, or 200 mg of calcium disodium EDTA per kg of body weight per day for 12 months. At the end of the study, all dogs appeared to be well, and there were no significant changes in blood or urine analysis. Gross and microscopic examinations of the major organs were normal (Oser et al., 1963).

Long-term exposure

The long-term toxicity of EDTA is complicated by its ability to chelate essential and toxic metals, both in water and in animals. Toxicity data are therefore equivocal and difficult to interpret.

An early study by Krum (1948) demonstrated no adverse effect on weight gain, appetite, activity, and appearance in rats fed for 44–52 weeks on a diet containing 0.5% disodium EDTA.

A 2-year study was carried out in which groups of rats were fed 0, 0.5, 1, or 5% disodium EDTA. The highest dose group showed a reduced food intake compared with the other groups and also suffered diarrhoea. No significant effects on weight gain were noted, nor were blood coagulation time, red blood cell counts, or bone ash adversely affected. Mortality of the animals could not be correlated with the level of disodium EDTA, as the highest mortality was observed in the control group and was due to pneumonia. Gross and microscopic examinations of the major organs did not reveal any significant differences between the groups (Yang, 1952).

In another study, groups of 25 male and 25 female rats were fed diets containing 0, 50, 125, or 250 mg of calcium disodium EDTA per kg of body weight per day for 2 years, and the study was carried on through four successive generations. The rats were mated after 12 weeks of feeding and were allowed to lactate for 3 weeks, with 1 week's rest before producing a second litter. Ten male and 10 female rats from the F_1 generation and similar F_2 and F_3 generations were allowed to produce two litters. With the second litter in the F_1, F_2, and F_3 generations, only the control and the 250 mg/kg of body weight per day dose groups were

kept until the end of the 2-year study on the F_0 generation. No significant abnormalities in appearance or behaviour were noted during the 12 weeks of the post-weaning period in all generations. The experiments showed no statistically significant differences in weight gain, food efficiency, haematopoiesis, blood sugar, non-protein nitrogen, serum calcium, urine, organ weights, and histopathology of the liver, kidney, spleen, heart, adrenals, thyroid, and gonads (Oser et al., 1963).

Fifty weanling albino rats were fed a low-mineral diet (0.54% calcium and 0.013% iron) with the addition of 0, 0.5, or 1% disodium EDTA or 0.5 or 1% calcium disodium EDTA for 205 days. Diarrhoea was observed in the 1% disodium EDTA group, along with other abnormalities: growth retardation of the males, lowered erythrocyte and leukocyte counts, a prolonged blood coagulation time, slightly but significantly raised blood calcium level, a significantly lower ash content of the bone, and considerable erosion of the molars. Gross and histological examinations of the major organs revealed nothing abnormal. Rats fed for 220 days on an adequate mineral diet containing 1% disodium EDTA showed no evidence of dental erosion (Chan, 1956).

Groups of 50 male and 50 female $B6C3F_1$ mice received trisodium EDTA in the diet at concentrations of 3, 750, or 7500 mg/kg of feed for 103 weeks, followed by 1 week during which the mice were fed standard diet without EDTA. The animals were examined twice per day for signs of toxicity. Gross and histopathological examinations of major organs and tissues were performed on animals found dead or moribund and on those sacrificed at the end of the study. Body-weight gain was decreased in high-dose males during the second year of the study, although no statistical analysis was presented. No treatment-related tumours or non-neoplastic lesions were observed in this study (NCI, 1977).

Reproductive and developmental toxicity

Groups of six rats were maintained on diets containing 0.5, 1, or 5% disodium EDTA for 12 weeks. The only toxic symptoms observed were diarrhoea and a reduction in food consumption at the 5% level. When the animals were 100 days old, mating was carried out and was repeated 10 days after weaning of the first litters. The animals given 5% disodium EDTA did not produce any litters. The other dose groups produced normal first and second litters (Yang, 1952).

Oser et al. (1963) carried out a four-generation study in which groups of rats received calcium disodium EDTA at doses of 50, 125, or 250 mg/kg of body weight per day via the diet. There were no reproductive or teratogenic effects noted in any of the three generations of offspring.

Groups of pregnant Sprague-Dawley rats were given diets containing 2 or 3% disodium EDTA from day 1 through day 21 of gestation. A further group of pregnant rats was fed diets containing 3% disodium EDTA from day 6 to day 14 of gestation, whereas a third group was given diets containing 3% disodium EDTA and 1000 mg of zinc per kg of feed from day 6 to day 21 of gestation. The control animals received a standard diet that contained 100 mg of zinc per kg of feed. In rats fed the 2% disodium EDTA, litter size was normal and fetuses were alive. Gross abnormalities were evident in 7% of the treated fetuses, compared with 0% in the control group. In rats fed 3% disodium EDTA, almost half of the implantation sites had dead fetuses or resorptions. Full-term young were significantly smaller than controls, and 100% of them were malformed. Maternal toxicity was indicated by diarrhoea and was observed in both the 2% and 3% dose groups. The malformations included severe brain malformations, cleft palate, malformed digits, clubbed legs, and malformed tails. Supplementation of the diet with 1000 mg of zinc per kg of feed prevented these detrimental effects, and it was suggested that the teratogenic effects of EDTA given to rats at very high levels were due to zinc deficiency (Swenerton & Hurley, 1971).

Schardein et al. (1981) performed teratogenesis studies with EDTA and its salts in rats. EDTA (967 mg/kg of body weight), disodium EDTA (1243 mg/kg of body weight), trisodium EDTA (1245 mg/kg of body weight), tetrasodium EDTA (1374 mg/kg of body weight), and calcium disodium EDTA (1340 mg/kg of body weight) were administered orally (by intubation) to groups of 20 inseminated female rats during organogenesis. The dosing regimen was twice daily on days 7–14 of gestation. There were two control groups. One group received 1.0 ml of phosphate buffer per kg of body weight twice daily to serve as a vehicle control group, and the other remained untreated and served as an untreated control group. The dams were killed on day 21 of gestation, and litter data for each dam were collected. The fetuses were then examined for gross external anomalies, visceral abnormalities, and skeletal anomalies. Diarrhoea was apparent in all the treated groups, and reduced activity was observed in a few of the dams. The diarrhoea generally occurred following treatment and disappeared on the last day of dosing or the day after. Three dams died during treatment with disodium EDTA. Examination of these did not indicate any gross abnormalities. Food intake was slightly reduced compared with controls during the treatment period of days 7–14 but was comparable to the controls during the pre-treatment period (gestation days 0–7) and post-treatment period (gestation days 14–21). None of the test compounds significantly affected litter size at term when compared with either control group. The mortality index of the offspring in all treated groups as measured by post-implantation loss was also comparable to both control groups. A total of 24 pups from the treated groups had abnormalities, including bifid vertebrae, agenesis of the ribs, inhibition of osteogenesis of the skull or ribs, and malformed ribs. There was, however, no pattern between treatment with any particular compound and the appearance of anomalies. In addition, the untreated control group had eight pups with some major defect. The results of these studies demonstrated that there was an absence of teratogenic effects even at doses that were maternally toxic.

An important study was carried out by Brownie et al. (1986), who investigated the teratogenic effect of calcium EDTA in rats and the protective effect of zinc. Pregnant Long-Evans rats were randomly assigned to 11 treatment groups corresponding to differing doses of calcium EDTA (2, 4, 6, or 8 mmol/m^2 per day), zinc EDTA (8 or 20 mmol/m^2 per day), and zinc calcium EDTA (8 or 20 mmol/m^2 per day), plus controls. Each group contained 20 rats, except for the control group receiving 0.9% NaCl, which contained 30 rats. A further 12 animals per group were used for maternal plasma and liver and fetal zinc analysis. The rats were treated by subcutaneous injection of the chelating agent or saline solution on days 11 through 15 of gestation. Results showed increases in several abnormalities (e.g. submucous cleft, cleft palate, curly tail, abnormal rib and vertebrae) with increasing doses of calcium EDTA. No malformations were seen with zinc EDTA at either dose or with zinc calcium EDTA at the lower dose. However, submucous cleft was seen in 6 of 20 litters from the dams receiving the higher dose of zinc calcium EDTA. It was concluded that calcium EDTA is teratogenic in rats at concentrations that, except for decreased weight gain, produce no discernible toxicity to the dam, and that protection is afforded by incorporating zinc in the chelate.

A study was carried out by Kimmel (1977) in which groups of pregnant CD rats were treated with disodium EDTA via a number of different routes of exposure. Forty-two rats received a dose of 954 mg/kg of body weight per day via the diet. Twenty-two received a dose of 1250 mg/kg of body weight per day, which was split into a dose of 625 mg/kg of body weight twice per day by gastric intubation. Eight rats received 1500 mg/kg of body weight per day as a split dose of 750 mg/kg of body weight twice per day by gastric intubation. Twenty-five rats received a dose of 375 mg/kg of body weight per day by subcutaneous injection. All the animals were dosed on gestation days 7–14. Fetuses were removed on day 21 of gestation and examined for gross and visceral abnormalities. The results showed that for the dietary group, there were no maternal deaths but a significant increase in fetal death compared with the controls, and 71% of the fetuses were malformed. In the group that was administered 625 mg/kg of body weight per day by gavage, only 64% of the dams survived treatment. Those

that survived exhibited fetal resorptions comparable to controls, and 20.5% of the fetuses were malformed. In the group administered 750 mg/kg of body weight per day, seven of the eight dams died. The group receiving subcutaneous injection of disodium EDTA showed a 76% survival of the dams. However, there was a significant increase in fetal resorptions compared with controls, although the proportion of malformed fetuses was similar to the controls. This study demonstrates that the route of exposure to EDTA is an important factor in the determination of the toxicity and teratogenic potential of EDTA.

Mutagenicity and related end-points

Trisodium EDTA was tested for its mutagenic potential in the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay. Two experiments were performed with S9 metabolic activation system and three without S9 at concentrations of EDTA up to 5000 mg/litre. No mutagenicity was observed either with or without the S9 (McGregor et al., 1988).

Trisodium EDTA was also tested in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 and in *Escherichia coli* WP uvrA in the presence and absence of the S9 metabolic activation system. There was no evidence of any mutagenic potential in any of these bacterial systems (Dunkel et al., 1985).

Carcinogenicity

In an experiment in which groups of 50 male and 50 female $B6C3F_1$ mice received trisodium EDTA in the diet at concentrations of 3, 750, or 7500 mg/kg of feed for 103 weeks, followed by 1 week during which the mice were fed standard diet without EDTA, no treatment-related tumours were observed (NCI, 1977).

GUIDELINE VALUE

JECFA evaluated the toxicological studies available on sodium iron EDTA in 1993 (WHO, 1993), and there was no further information, compared with its 1973 evaluation (WHO, 1974), of noteworthy importance regarding the toxicity of EDTA and its calcium/sodium salts. Concern has been expressed over the ability of EDTA to complex zinc and therefore reduce its availability. However, this is only of significance at elevated doses substantially in excess of those encountered in the environment. The use of an additional uncertainty factor and the assumption of a 10-kg child were therefore considered inappropriate.

A guideline value for EDTA in drinking-water can be derived by allocating 1% of the JECFA ADI (1.9 mg/kg of body weight as the free acid) to drinking-water (because of the potential for significant exposure from food owing to its use as a food additive). Therefore, assuming a 60-kg adult ingesting 2 litres of drinking-water per day, the guideline value for EDTA (free acid) is 600 µg/litre (rounded figure). This value is no longer considered to be provisional.

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