European Journal of Nutrition & Food Safety
4(4): 408-423, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Short Review of Calcium Disodium Ethylene Diamine Tetra Acetic Acid as a Food Additive

Marijke M. H. Van De Sande[1], Sabrina Wirtz[1], Ellen Vos[1] and Hans Verhagen[2,3*]

[1]Maastricht University, Department of Human Biology, PO Box 616, 6200 MD Maastricht, The Netherlands.
[2]National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands.
[3]University of Ulster, Northern Ireland Centre for Food and Health (NICHE), Cromore Road, Coleraine, BT52 1SA Northern Ireland.

Authors' contributions

This work was carried out in collaboration between all authors. Authors HV and SW designed the work. Author MHVDS conducted the literature research, analyzed the data and wrote the first version. Authors HV, SW and EV were responsible for subsequent reviewing and scientific editing, while author MHVDS was the primary responsible for final content. All authors read and approved the final manuscript. The authors declare no conflicts of interest and this research received no grant from any funding agency.

Mini Review Article

Received 26th March 2014
Accepted 24th June 2014
Published 14th July 2014

ABSTRACT

Calcium disodium ethylenediaminetetraacetate (Calcium Disodium EDTA, $C_{10}H_{12}CaN_2Na_2O_8.2H_2O$) is a derivative of EthylenediamineTetraacetic Acid and is an approved food additive (E385). It is used as preservative, sequestrant, flavouring agent, and colour retention agent in foods. As a drug it is used for the reduction of blood and mobile depot lead in the treatment of acute and chronic lead poisoning. Calcium Disodium EDTA is very poorly absorbed from the gastrointestinal tract following ingestion. The compound is metabolically inert and no accumulation in the body has been found. Acute, short-term, sub chronic and chronic toxicity studies carried out with Calcium Disodium EDTA in laboratory animals found that the compound is nephrotoxic at high doses. In similar high doses, application of Calcium Disodium EDTAcan result in complexation of zinc ions, thus interfering with the zinc homeostasis and causing developmental toxicity.

**Corresponding author: Email: Hans.verhagen@rivm.nl;*

No evidence exists suggesting the compound exerts genotoxic or carcinogenic effects. Overall, Calcium Disodium EDTAseems to be safe for use as a food additive, as the noted toxic doses are higher than can be achieved via the addition of Calcium Disodium EDTA to food. However, human data is limited and the gross of available (human and animal) data, as well as the ADI, stems from several decades ago. Caution should also be taken when Calcium Disodium EDTA is administered as treatment for lead poisoning, as the exposure increases greatly. Until 2020, EFSA will carry out new risk assessments, and subsequently the Commission will revise the list of food additives and the conditions of use specified therein. The deadline for food additives other than colours and sweeteners is 31 December 2018, which seems appropriate regarding the non-acute need for re-evaluation of Calcium Disodium EDTA as food additive.

Keywords: Food additives; calcium disodium ethylenediaminetetraacetate; EDTA; CaNa2EDTA; lead poisoning; toxicological evaluation.

1. INTRODUCTION

Calcium disodium ethylenediaminetetraacetate (Calcium Disodium EDTA) is a derivative of EthylenediamineTetraacetic Acid (EDTA). EDTA and its salts (known collectively as Edetates) have uses in foods, pharmaceutical products, and manufacturing, and are used to treat heavy metal poisoning [1]. Calcium Disodium EDTA was even found to be able to decrease plasma cholesterol concentrations in hypercholesterolemic patients. In addition, some salts function as chelating agents in cosmetic formulations, where they combine with polyvalent metal cations in solution in order to form soluble ring structures [2].

Calcium Disodium EDTA consists of white, odourless, crystalline granules, with a faint, salty taste[3]. In the food industry, it is commonly used a preservative and sequestrant. It is sometimes also referred to as Calcium Disodium Edetate or $CaNa_2EDTA$.

In this short review paper, the available evidence regarding the chemical, biological and toxicological aspects of Calcium Disodium EDTA as a food additive is elaborated and discussed. First, the chemical properties are described, then the fate of Calcium Disodium EDTA in foods, the intake, and the legal status are described, next the existing biological data will be discussed, including absorption, distribution, metabolism and excretion, and other effects of Calcium Disodium EDTA in the human body are described, and finally, the available evidence regarding the possible toxicity of Calcium Disodium EDTA will be elaborated.

2. CHEMICAL PROPERTIES

2.1 Chemical Structure and Identity

The molecular formula of Calcium Disodium EDTA is $C_{10}H_{12}CaN_2Na_2O8.2H_2O$, and its structural formula is given in Fig. 1. The molecular weight is 410.31[3] and the chemical identity CAS No. 23411-34-9.

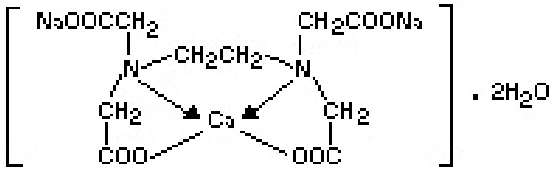

Fig. 1. Structural formula of calcium disodium EDTA

Calcium Disodium EDTAis soluble in water such that a 0.1 M solution (pH = 7) can be prepared at 30ºC. It is "practically insoluble" in organic solvents. The pH of a 1:5 or 1:100 solution is 6.5 to 8.0. The molecular weight is 374.27 (anhydrous) or 410.3 Da (2aq) and the melting point is >300ºC [4,5].It is stable in air [4]. EDTA, the Edetates, HEDTA, and Trisodium HEDTA are chelating agents. They are neutralized by alkali-metal hydroxides to form water-soluble salts, or chelates, that contain metal cations [6,7]. Food- and pharmaceutical-grade Calcium Disodium EDTA is a mixture of the dihydrate and trihydrate of Calcium Disodium EDTA (predominantly the dihydrate), and contains not less than 97.0% and not more than 100.2% of $C_{10}H_{12}CaN_2Na_2O_8$, calculated on the anhydrous basis [1,4,5]

2.2 Manufacturing Process

Industrial methods to manufacture EDTA salts involve the mixing of formaldehyde and hydrogen cyanide or an alkali metal cyanide to form an aqueous solution of EDTA. The saltsare then formed by hydrolysis [8]. EDTA can also be formed by heating tetrahydroxyethylethylenediamine with sodium or potassium hydroxide using a cadmium oxide catalyst [6]. Calcium Disodium EDTA can be produced by boiling an aqueous solution of Disodium EDTA with slightly more than an equimolar quantity of calcium carbonate until carbon dioxide no longer evaporates. The resulting solution is filtered while hot and crystallized [1,4]. Disodium EDTA can be prepared by dissolving EDTA into a hot solution that contained two equivalents of sodium hydroxide [4].

3. CALCIUM DISODIUM EDTA AS FOOD ADDITIVE

3.1 Uses in Foods, Reaction and Fate in Food

EDTA is an antioxidant [9], and Calcium Disodium EDTA is a preservative, sequestrant [5], flavouring agent, and colour retention agent [7]. Currently, EDTA and the EDTA salts are used for their wide number of functions, such as maintaining clarity, protecting fragrance components, stabilizing polymeric thickeners and colour additives, preventing rancidity, and increasing preservative effectiveness [10]. More specifically, EDTA and its salts prevent discoloration due to trace metals in antibiotics, antihistamines, and local anaesthetics. They are used to stabilize solutions of ascorbic acid (vitamin C), hydrogen peroxide, formaldehyde, folic acid and hyaluronidase. These ingredients are also used in detergents and agricultural chemical sprays; in metal cleaning, etching, cutting, and plating operations; for the decontamination of radioactive surfaces; as an eluting agent in ion-exchange reactions; as a bleaching agent in colour in processing; and in analytic chemistry and spectrophotometric titrations [6,8].

Clinically, EDTA and Calcium Disodium EDTA are used for the diagnosis and treatment of heavy metal poisoning, including exposure to lead, vanadium, and cadmium [11-13].

Calcium Disodium EDTA is also added to pharmaceuticals to prevent calcium depletion in the body [7].

3.2 Exposure/ Intake

At present, the only exposure to EDTA from foods in the European market is from Calcium Disodium EDTA in foods, such a scanned and bottled crustaceans and molluscs, canned and bottled fish, emulsified sauces, and canned and bottled pulses, legumes, mushrooms and artichokes [14]. Based on the approved use levels of Calcium Disodium EDTA and the food consumption data from the UK National Diet and Nutrition Survey, the daily intake of EDTA was calculated to be in the range of 2.4 mg/day for young children to 4.8 mg/day for adult males on average and 8.7 mg/day to 15.8 mg/day, respectively, at the 95th percentile. This is equivalent to 0.2 mg/kg bw/day and 0.06 mg/kg bw/day on average and 0.6 mg/kg bw/day and 0.2 mg/kg bw/day at the 95th percentile [15]. It has been estimated that daily human consumption of Calcium Disodium EDTA in the United States is0.23mg/kg bw/day at the 90^{th} percentile [16].

3.3 Legal Status in EU and Beyond

Calcium Disodium EDTA is the only currently approved EDTA derivative for food use in the EU, under the E-number E385 [14]. The additive is authorised to be used in spreadable fats having a low fat content (ML = 100 mg/kg), canned or bottled pulses, legumes, mushrooms and artichokes (ML = 250 mg/kg), specific heat treated processed meat (ML = 250 mg/kg), frozen and deep-frozen crustaceans (ML = 75 mg/kg), canned and bottled fish, crustaceans and molluscs (ML = 75 mg/kg), and emulsified sauces (ML = 75 mg/kg) (Annex II of Regulation (EC) No 1333/2008). In the US, Calcium Disodium EDTA can be used as a food additive and is permitted for direct addition to food for human consumption, as long as 1) the quantity of the substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritive, or other technical effect in food, and 2) any substance intended for use in or on food is of appropriate food grade and is prepared and handled as a food ingredient (21 CFR 172.120).

The daily intake level causing no toxicological effect in rats was established by studies finding a level of 50 000 ppm in the diet, equivalent to 250 mg/kg bodyweight/day. From here the estimates of acceptable daily intakes for man were calculated and established by JECFA. In 1974, the JECFA evaluated Calcium Disodium EDTA as a food additive and derived an ADI for this substance of 0-2 mg/kg bw/day [17]. In 1977 and in 1990, the Scientific Committee for Food (SCF) evaluated Calcium Disodium EDTA as an antioxidant and endorsed the ADI established by the JECFA [18,19].

The use of Calcium Disodium EDTA and disodium EDTA as direct additives to foods is also permitted in North and South America, Asia (e.g., Malaysia, Philippines), Africa, and Australia [20,21].

3.4 Future Risk Assessments

With introduction of the legislative Package on Food Improvement Agents in December 2008 also the regulation of food additives in the European Union was reformed. Regulation 1333/2008 of the European Parliament and the Council, introduced one regime for the use of food additives in the Union, food colours sweeteners and the remaining food additives that

until then were regulated in separate Directives (respectively Directive 94/36/EC, Directive 94/35/EC and Directive 95/2/EC). Apart from consolidating the regulation of food additives, Regulation 1333/2008 also started a process of re-evaluation of all food additives authorized for use in the Union prior to 20 January 2009 (see Commission Regulation No 257/2010). Consequently, until 2020, EFSA will carry out new risk assessments for these additives, taking into account scientific literature, the original scientific information and data provided to the Authority through calls for data in which EFSA calls upon the Member States and other stakeholders to provide specific data sets. Based on the re-evaluation carried out by EFSA, the Commission will revise the list of food additives and the conditions of use specified therein. Hereby it will start with the revision of food colours (deadline 31 December 2015), followed by food additives other than colours and sweeteners (31 December 2018) and sweeteners (31 December 2020).

4. BIOLOGICAL DATA

4.1 Absorption, Distribution, Metabolism and Excretion

Calcium Disodium EDTA is very poorly absorbed from the gastrointestinal tract following ingestion by humans as well as animals. EPA (2006) indicated an upper limit of absorption of 20%, with elimination occurring primarily via the kidneys (95%) [22]. Calcium Disodium EDTA is well absorbed following IM or subcutaneous administration [23].

The distribution of Calcium Disodium EDTA occurs mainly into extracellular fluid with only 5% of the plasma concentration found in spinal fluid [24].The compound is metabolically inert and no accumulation in the body has been found. Several studies in rats concluded that almost the entire intraperitoneally, intravenously, intramuscularly and/or orally administered dose was eliminated from the body within 24 to 48 hours of dosing [1,25,26].

Experiments in humans also revealed poor absorption of Calcium Disodium EDTA [27,28]. Only 2.5% of a 3-g oral dose was excreted in the urine [27]. Foreman and Trujillo [23] treated healthy adult males (3 per group) with ^{14}C-Calcium Disodium EDTA to determine its metabolism in humans after topical, intravenous and intramuscular administration. The authors concluded that Calcium Disodium EDTA passed through the body unchanged, and was excreted almost entirely through the kidneys by both glomerular filtration and tubular excretion. Absorption from the GI tract occurred at a maximum of 5% of the dose. The half-life of Calcium Disodium EDTA in blood was approximately one hour after intravenous administration and one and a half hours after intramuscular injection. The only apparent interaction with the body was the combination of EDTA with di- and tetravalent metal ions [23]. Intravenous doses of 3 g of ^{14}C-labelled Calcium Disodium EDTA, given to two subjects, were almost entirely excreted within 12–16 hours [27]. After both intravenous and intramuscular administration of Calcium Disodium EDTA in humans >98% of the dose was excreted unaltered primarily in the urine [23].

4.2 Treatment of Metal Poisoning

Calcium Disodium EDTA as a drug is used for the reduction of blood and mobile depot lead in the treatment of acute and chronic lead poisoning and the management of acute lead encephalopathy and symptomatic lead poisoning [29]. It is used in conjunction with the drug Dimercaprol since Calcium Disodium EDTA alone may aggravate manifestations of toxicity in patients with very high blood lead concentrations [29].

Also EDTA itself and other EDTA-salts are so called chelating agents. They are strongly attracted to alkaline earth and transition metal ions. During a reaction with EDTA, the metal ion is converted to an anionic form as part of a metal-EDTA complex; thus, the oxidation-reduction potential of the metal ion is altered and the partitioning of the metal to the aqueous phase is enhanced [10]. The chelating action of EDTA occurs at alkaline pH as long as metallic ions are available, until all the EDTA molecules are utilized. One mole of EDTA chelates one mole of metallic ions [30]. The 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 [10,30,31]. Calcium Disodium EDTA will chelate any other metal that has a higher binding affinity than calcium (e.g. lead, iron, zinc, and copper) [32]. Lead chelates with Calcium Disodium EDTA to form a complex that is 10^7 times stronger than that of the calcium complex. However, zinc shows a 10^4 times higher binding affinity than that of the calcium complex. Also, application of Calcium Disodium EDTA will result in complexation of zinc ions, thus interfering with the zinc homeostasis and leading finally to developmental toxicity [32]. This process will be elaborated below.

A 10-fold increase in urinary excretion of zinc during the administration of Calcium Disodium EDTA was found in 24-hour urine samples from hypercholesterolaemic patients. A smaller effect on cadmium, lead, vanadium, and manganese may have occurred, although these results were not clear [33]. Furthermore, it was reported that EDTA enhanced the excretion of cobalt, cadmium, calcium, mercury, manganese, nickel, lead, thallium, and tungsten [34,35], although findings vary across studies. Allain et al. [36] infused 10 healthy subjects with Calcium Disodium EDTA intravenously over 1 h, to measure the change in 24 h urinary elimination of several elements. The following ratios were reported for the increase of urinary elimination: About 2for Iron (thus about two times higher excretion of iron after 1h Calcium Disodium EDTA infusion compared to before infusion), 5 for aluminium, lead and manganese, and 15 for zinc [36]. As the increase in zinc excretion could lead to a deficiency, zinc supplements should be considered in patients treated by successive infusions of Calcium Disodium EDTA.

Injections of 75 or 150 mg/kg Calcium Disodium EDTA for 1, 2, 3, 4 or 5 days decreased the level of lead in blood in rats, although values never reached the range of non-exposed controls. No additional reduction in blood lead level was produced after the first injection of Calcium Disodium EDTA. Urinary lead excretion increased substantially after Calcium Disodium EDTA injections, as compared to the controls. However, brain lead was significantly increased after one injection due to internal redistribution of lead [37]. Foreman [35] has concluded that the maximal safe dose of Calcium Disodium EDTA in humans is 75 mg/kg bw/24 hours. As this amount is attended with some risk, the author advises that the dose of 50 mg/kg bw/24 hours not be exceeded in the usual case of acute plumbism. Couses should not exceed 5 to 7 consecutive days, with an interval of at least 2 days between courses. Long-term oral administration of Calcium Disodium EDTA in plumbism is not warranted [35].

Some randomized controlled trials in patients with chronic lead poisoning, coronary artery disease and chronic renal insufficiency showed very few adverse effects for a prolonged chelation treatment with a low dose of Calcium Disodium EDTA (40 mg/kg bw per week or less for a duration of 33 days to 24 months)[13,38-40].However, studies using higher doses to treat acute lead poisoning did report serious adverse effects of chelation therapy in humans. Reuber and Bradley [41] reporteda 38-year-old man with pre-existing renal disease, who died after developing acute tubular necrosis complicated by pneumonia after receiving 600 rather than 60 mg/kg/day of sodium calcium EDTA for 5 days as treatment for

lead intoxication. In addition, Moel and Kumar [42] treated 130 children (1-8 yrs.) with chelation therapy (dimercaprol and Calcium Disodium EDTA, 50 mg/kg/day for 5 days) for asymptomatic lead poisoning. 16% of the children had biochemical evidence of transient nephrotoxicity and 3% had acute oliguric renal failure.

5. TOXICOLOGICAL DATA

The Calcium Disodium EDTA complex consists of Calcium Disodium EDTA species and free anionic EDTA species in solution. Although EDTA itself generally has toxic effects on the human body upon ingestion, the EDTA species in Calcium Disodium EDTA comprise only <0.01% of the complex according to the mass action law. Therefore, the WHO considered this amount to be too low for detecting generally toxic effects of EDTA or Na_4EDTA in Calcium Disodium EDTA [32].

5.1 Acute Toxicity

Acute toxicity studies have been carried out with disodium EDTA and Calcium Disodium EDTA in laboratory animals. The acute oral ld_{50} of Calcium Disodium EDTA for rats was found to be 10.0±0.74 g per kg bodyweight and for the rabbit and dog, approximately 7 and 12 g, respectively. The acute toxicity in rats was not altered by prior feeding of a diet suboptimal in respect to calcium, iron, copper, and manganese [43]. In another study, the acute intraperitonealLD_{50} of Calcium Disodium EDTA in mice, rats and rabbits exceeded 4.5, 7, and 6 mg/kg, respectively [44].

Schwartz et al. [45] administered Calcium Disodium EDTA intraperitoneally in single doses of 1.0 or 2.5 g/kg to rats. After 2 hours, no biochemical changes were found, although there was some vacuolization of predominantly the tubular part of the outer cortex of the kidney. After 24 hours, there were definite biochemical changes at both doses. However, there were no significant decreases in the renal cortical content of zinc, iron, manganese, copper, magnesium, cobalt, or calcium noted at 24 hours, and within 6-24 hr, virtually the entire administered dose has been excreted in the urine. Therefore, it could be that Calcium Disodium EDTA had little if any direct effect on the biochemical enzymes studied, for no significant changes were noted in the 2-hr specimens, nor were the enzyme systems affected by addition of Calcium Disodium EDTA *In vitro* [45].

Approximately one year later, the same research group [46] dosed male, albino, Sprague-Dawley rats with an intravenous injection of 10, 100, and 1000 mg/kg radioactive Calcium Disodium EDTA. The kidneys were removed at 0.5, 1, 2, 3, 6, and 24 hours after injection; activity of the radioactive chelate was found in the renal cortex 30 minutes to 24 hours after dosing that decreased with time. The peak concentration of Calcium Disodium EDTA appeared in the cortical sediment within 30 minutes at all three doses. The cortical supernatant had a sharp decrease in activity over the first 2 to 3 hours, whereas the cortical sediment activity did not decrease until after 6 hours [46].

Ahrens and Aronson [47] intravenously administered Calcium Disodium EDTA to female mongrel dogs, at 1.5 mmol/kg bw/day(approximately 438 mg/kg bw/day) continuously for 48 hours. They found signs of toxicity, a decreased glomerular filtration rate, decreased renal plasma flow, and decreased tubular maximafor glucose and PAH in the treated dogs after 36 hours. In addition, histopathologic changes, a marked reduction of glucose absorption from the duodenum and an increased permeability of the intestine were found. No changes

occurred in blood urea nitrogen, plasma electrolyte concentrations, or urinary excretion rates of sodium, potassium, or chloride. The authors concluded that Calcium Disodium EDTA toxicity was due to its chelating properties rather than some nonspecific action of the EDTA moiety [47].

Araki et al. [48] observed an average 11-fold increase in 24-h urine zinc excretion in workers exposed to lead after a single intravenous infusion of 20 mg Calcium Disodium EDTA/kg bw, but no evidence of associated clinical effects.

5.2 Short Term Toxicity

Kawamata et al. [49] compared the toxicities of 1% to 5% Disodium EDTA and 5.5% Calcium Disodium EDTA in a 1-month feeding study in rats. They found marked suppression of body weight gain, decreased leucocyte and lymphocyte counts, decreased calcium serum concentration, microscopic changes, and decreased weights of the liver, spleen and thymus for Disodium EDTA. Similar but milder effects were observed in rats treated with Calcium Disodium EDTA[49]. Drinking water with added Calcium Disodium EDTA orally administered to mice was found to decrease hepatic calcium and renal magnesium concentrations [50].

In a 30-day study using female rats, the intraperitoneal LD_{50} was estimated at 3800 mgCalcium Disodium EDTA/kg bw/day. The rats were treated with 500 to 7000 mg/kg in three doses at 2, 4, and 6 hours during a 24 hour feeding period [51]. It was reported that in male rats, weight gain remained satisfactory and that the histology of the lung, thymus, liver, spleen, adrenal, small gut, and heart remained normal after IP administration of 250 or 500 mg of Calcium Disodium EDTA/kgbw/day for 3–21 days; there was only a mild to moderate effect on the kidney [52]. Many studies evaluated the short-term nephrotoxic effects of intraperitoneal Calcium Disodium EDTA administration in rats. Most of them found a decrease in bodyweight and microscopic changes varying from small vacuoles in the epithelial cells of the proximal tubules, to extrusion of cell contents through the disrupted apical border, compared to controls, and lesions (e.g. parenchymatous degeneration in the distal tubules, amorphous casts in the collecting tubules) within 48 hours in animals that received a high dose (3000 mg/kgbw/day) [25,53]. Reuber et al. found in a series of studies [54-56], which lasted 21 days, that Calcium Disodium EDTA–induced lesions in rats are a function of age.

Male Long-Evans rats that were intravenously infused with 6 mmol or 1753 mg Calcium Disodium EDTA/kg bw/day for 48 hours showed significantly lower total 5-HT concentrations in brain, duodenum, and kidney [57]. According to the authors, depletion of brain 5-HT may be related to metal chelation, as zinc ions may regulate the level of serotonin and norepinephrine content in the brain [58], whereas 5-HT depletion in the duodenum could be due to damage to the duodenal mucosa [57]. Male albino rats treated with the same amount of Calcium Disodium EDTA/kg bw/day for 72 hours developed marked depletion of collagen fibrils in the skin, indicating that Calcium Disodium EDTA enhances collagen degradation in the rat [59]. Male sprague-dawley rats that were parentally infused continuously with 12 mmol/kg(or 3506.88 mg Calcium Disodium EDTA/kg bw) over a 48-hour period became depressed and had diarrhoea and blood in their urine by the end of the treatment. In addition, the intestinal wall was thin, the lumen was filled with gas, and the intestinal weight was significantly less than that for control animals. Increased excretion of certain amino acids occurred 36 hours following infusion in rats that had blood in the urine [60]. In time response experiments [61] rats received Calcium Disodium EDTA at dosages of 0.75, 1.5, 3

and 6mmol/kg bw/24 hours (equal to 219.18 to 1753.22 mg/kg) for 36 hours. Only the highest doseinduced morphological changes (e.g. shortening of the villi).

Human studies are limited, although adverse effects of Calcium Disodium EDTA as treatment for lead intoxication were reported, as described in chapter 4.2.

5.3 Subchronic Toxicity

A preliminary study in 1948 [62] 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. Oser et al. [43] fed four groups of one male and three female mongrel dogs diets containing 0, 50, 100, or 200 mg of Calcium Disodium EDTA /kg bw/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 [43].

Yang and Chan [63] fed groups of three albino rats per sex a low-mineral basal feed for 4 months. Rats of group 1 were fed basal diet only and rats of groups 2 to 4 were fed diet containing 0.5% disodium EDTA, 1.5% disodium EDTA, and 1.5% Calcium Disodium EDTA, respectively. Weight gain was significantly decreased in rats of groups 3 and 4, but no other signs of toxicity were observed [63]. Fifty weanling albino rats were fed a low-mineral diet with the addition of 0, 0.5, or 1% disodium EDTA or 0.5 or 1% Calcium Disodium EDTA for 90 days. Signs of diarrhoea and growth retardation were only observed in the 1% disodium EDTA dose group. Extension of dosing for an additional 115 days resulted in growth retardation in the groups fed diets containing 1.0% of each EDTA chelate [64]. Bibra reviewed the above mentioned sub chronic toxicity studies on Calcium Disodium EDTA and disodium EDTA and concluded that the presence of minute quantities of Calcium Disodium EDTA (10-100 ppm) in foods would not be expected to have any significant effect on health, and that an adult diet containing as much as 4000 ppm EDTA would still be far less than the lowest quantity fed to rats (0.5% in the diet) [65].

Daily intraperitoneal doses of 0.1, 0.2, 0.3, 0.5, 1.0 g Calcium Disodium EDTA/kg bw/day 5 days per week for 14 weeks to Wistar rats (6/dose) produced a graded weight reduction [1]. In a more recent study, calves were able to tolerate prolonged intraperitoneal infusion with 80 mg Calcium Disodium EDTA/kg bw twice a week over a 10 week-period as treatment for vanadium poisoning without side-effects [66].

Rabbits with experimental lead poisoning that were injected intravenously with 20 mg Calcium Disodium EDTA /kg bw/day on alternate days for 6 months had only slight changes in the blood and blood-forming organs [67].

5.4 Chronic Toxicity

Oser et al. [43] fed groups of 25 male and 25 female rats diets containing 0, 50, 125, or 250 mg Calcium Disodium EDTA /kg bw/day for 2 years, and the study was carried out on through four successive generations. Offspring were fed their respective parents' diets. 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 [43]. In the same paper, the authors report on dogs which were fed 50 to 250 mg Calcium Disodium EDTA/kg bw/day. Signs of adverse effects on growth or organ weights, osseous changes or other gross and microscopic lesions were not observed. Interestingly, hematologic findings suggested that all dogs treated with Calcium Disodium EDTA were even in a better state of health after 1 year than they were initially [43].

5.5 Genotoxicity and Carcinogenicity

In a review of available genotoxicity studies, Heindorff et al. [68] classified EDTA itself as a weak mutagen in microbial systems. However, Gentile, Hyde and Schubert [69] determined the mutagenicity of several chelates via Rec-assay, including Calcium Disodium EDTA (using *B. subtilis* and *S. typhimurium*). They found that Calcium Disodium EDTA was not only non-mutagenic, but also reduced the mutagenicity of $Cr_2O_3^{-2}$.

Other animal studies have not been conducted with Calcium Disodium EDTA to evaluate its carcinogenic potential, mutagenic potential or its effect on fertility [70]. In this regard, also EPA has concluded that there is no concern for EDTA with regard to carcinogenicity [22].

5.6 Reproductive and Developmental Toxicity

The four-generation study by Oser et al. [43] previously mentioned, in which groups of rats received Calcium Disodium EDTA at doses of 50, 125, or 250 mg/kg bw/day via the diet, found no reproductive or teratogenic effects in any of the three generations of offspring.

Brownie et al. [71] evaluated the teratogenic potential of Calcium Disodium EDTA in pregnant Long-Evans rats with differing doses of Calcium Disodium 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. Rats who were fed 4mmol/m^2Calcium Disodium EDTA had decreased weight gain and rats with higher doses had diarrhoea as well as decreased feed consumption, water intake, urine production, weight gain, and mild renal lesions. Results showed increases in several abnormalities (e.g. submucous cleft, cleft palate, curly tail, abnormal rib and vertebrae) in the litters with increasing doses of calcium EDTA. In this study, maternal and foetal toxicity were not observed for groups treated with Zinc EDTA or the low dose of Zinc Calcium EDTA. The investigators concluded that Calcium EDTA was teratogenic to rats at concentrations that, except for decreased weight gain, produce no discernible toxicity to the dam, and that zinc had a protective effect when incorporated into the chelate [71].

Schardein et al. [72] orally administered EDTA, Disodium EDTA, Trisodium EDTA, Tetrasodium EDTA, and Calcium Disodium EDTA twice daily to groups of 20 inseminated female rats on days 7–14 of gestation. There were two control groups: one vehicle control group that received phosphate buffer, and one untreated control group. Diarrhoea was apparent in all the treated groups, and reduced activity was observed in a few of the dams. The test compounds did not significantly affect litter size, post implantation loss, or the sex ratio compared to controls. A total of 24 pups from the five treated groups, regardless of the particular compound, had abnormalities, including bifid vertebrae, agenesis of the ribs, inhibition of osteogenesis of the skull or ribs, and malformed ribs. However, also the untreated control group had eight pups with some major defects, such as bifid vertebrae, sternebrae, and eye defects. The results of these studies demonstrated that, despite the

appearance of abnormalities, there was an absence of a significant higher risk of teratogenic effects of Calcium Disodium EDTA or the other treatment groups compared to the control groups even at doses that were maternally toxic[72].

Apgar [73] reported that Calcium Disodium EDTA decreased the endogenous zinc supply in pregnant Long Evans and Sprague-Dawley rats by increasing the excretion of the metal. Female rats deprived of zinc from day 18 of gestation underwent stress at parturition. Stress, haematocrit parameters and the loss of body weight were increased when the females were also injected IP with 5 ml (0.1 M) Calcium Disodium EDTA, compared to females that were not given the chelating agent [73].

Two case reports are available of women treated for lead intoxication with Calcium Disodium EDTA and which delivered normal infants [74,75].

6. CONCLUSION

Calcium Disodium EDTA is currently widely approved and used as additive in several foodstuffs. Unlike Disodium EDTA, which has been widely used but currently removed from the market due to safety issues, Calcium Disodium EDTA seems to be safe for daily use under the described conditions. Animal toxicity studies have shown that Calcium Disodium EDTA is toxic only in high doses. In humans, these doses will certainly not be reached via the current level of food and environmental exposure.

No data suggest that there are any mutagenic or carcinogenic effects of Calcium Disodium EDTA. In addition, research has shown that there is virtually no absorption from the gastrointestinal tract after oral intake of the additive. As data from animal studies with Calcium Disodium EDTA did not provide evidence for adverse effects on reproductive performance and outcome for doses of up to 250 mg/kg bw/day, and no apparent sufficient human data is available, Calcium Disodium EDTA is not considered a reproductive toxicant, as long as zinc intake is sufficient. Caution, however, should be taken when Calcium Disodium EDTA is administered as treatment for lead poisoning, as the exposure increases greatly.

Overall, the little available evidence suggests that Calcium Disodium EDTA can still be safely used as a food additive. The highest reported exposure of 0.23 mg/kg bw/day does not come even close to the reported effective doses described above, such as 438 mg/kg bw/day for acute toxicity in dogs [47]. However, human data is limited and the gross of available (human and animal) data stems from several decades ago. Recent data that does exist, evaluating Calcium Disodium EDTA as chelating agent in chronic diseases or lead poisoning, show some adverse effects when administered intravenously in high doses (e.g. 50 mg/kg bw/day). In the majority of the cases, these effects seem to be caused by a depletion of essential minerals such as zinc. In addition, the ADI endorsed by the JECFA/SCF stems already from more than 2 decades ago. Therefore, the re-evaluation by EFSA, which will take place before 31 December 2018, for this important but non-acute issue, seems very appropriate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lanigan RS, Yamarik TA. Final report on the safety assessment of EDTA, Calcium Disodium EDTA, Diammonium EDTA, Dipotassium EDTA, Disodium EDTA, TEA-EDTA, Tetrasodium EDTA, Tripotassium EDTA, Trisodium EDTA, HEDTA, and Trisodium HEDTA. Int J Toxicol. 2002;21(2):95-142.
2. FDA. Frequency of use of cosmetic ingredients. FDA database. Washington, DC: FDA; 1998.
3. JECFA. Calcium disodium ethylenediaminetetraacetate. Geneva (Switz.): World Health Organization (WHO), 1966 Contract No.: 40A,B,C.
4. Gennaro AR. Remington's pharmaceutical sciences, 18th ed. Easton, PA: Mack Publishing Co.; 1990.
5. (NAS) NAoS. Food chemicals codex, 4th ed. Washington, DC: National Academic Press; 1996.
6. Lewis RJ, Sr. Hawley's condensed chemical dictionary, 12th ed. New York, NY: Van Nostrand Reinhold; 1993.
7. Budavari S. The Merck index: An encyclopedia of chemicals, drugs and biologicals, 11th ed. Rahway, NJ: Merck & Co; 1989.
8. HSDB. Hazardous Substances Database. Calcium Disodium EDTA, Tetrasodium EDTA, Trisodium EDTA, HEDTA, and Trisodium HEDTA. National Library of Medicine (NLM); 1998.
9. Entry on EDTA [Internet]. National Institutes of Health; 1998.
10. Hart JR. Chelating agents as preservative potentiators. In: Kabara JJ, editor. Cosmetic and drug preservation principles and practice. New York, NY: Marcel Dekker. 1984;323-37.
11. Sears ME. Chelation: Harnessing and enhancing heavy metal detoxification: A review. Sci World J. 2013;13.
12. Hu H. Heavy Metal Poisoning. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors. Harrison's Principles of Internal Medicine; 2012.
13. Besunder JB, Super DM, Anderson RL. Comparison of dimercaptosuccinic acid and calcium disodium ethylenediaminetetraacetic acid versus dimercaptopropanol and ethylenediaminetetraacetic acid in children with lead poisoning. J Pediatr. 1997;6//;130(6):966-71.
14. European Parliament and Council Directive No 95/2/EC of 20 February 1995 on Food Additives Other than Colours and Sweeteners; 1995.
15. EFSA. Scientific Opinion on the use of ferric sodium EDTA as a source of iron added for nutritional purposes to foods for the general population (including food supplements) and to foods for particular nutritional uses. In: EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Parma, Italy: EFSA; 2010.
16. Food Additive Safety profiles of Tetrasodium EDTA, Disodium EDTA, Calcium Disodium EDTA, and Dipotassium EDTA. [Internet]. FDA; 1998.
17. JECFA. Annex 4: Acceptable daily intakes [Ethylenediaminetetraacetate, disodium and calcium disodium salts]. Geneva (Switz.): World Health Organization (WHO); 1974. Contract No.: 539.

18. SCF. Report of the Scientific Committee for Food on a second series of food additives of various technological functions; 1990. Opinion expressed 19 October 1990. Available: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_26.pdf.
19. SCF. Report of the Scientific Committee for Food on calcium disodium ethylenediamine tetraacetate. Opinion expressed 24 June, 1977. SCF; 1977. Available: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_04.pdf.
20. Vierk K, Koehler K, Fein S, Street D. Prevalence of self-reported food allergy in American adults and use of food labels. J Allergy Clin Immunol. 2007;119:1504-10.
21. INACG. Iron EDTA for Food Fortification: A Report of the International Nutritional Anemia Consultative Group. Washington, DC: International Nutritional Anemia Cunsultative Group (INACG), Nutrition Foundation Inc.; 1993.
22. Environmental Protection Agency (EPA). Ethylendiaminetetraacetic acid chemicals: exemptions from the requirement of a tolerance. Federal Register. 2006;71(45):11563-11570.
23. Foreman H, Trujillo TT. The metabolism of C14 labeled ethylenediaminetetraacetic acid in human beings. The Journal of laboratory and clinical medicine. 1954;43(4):566-71.
24. Ziegler A, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA. 2003;290:1721-8.
25. Doolan PD, Schwartz SL, Hayes JR, Mullen JC, Cummings NB. An evaluation of the nephrotoxicity of ethylenediaminetetraacetate and diethylenetriaminepentaacetate in the rat. Toxicol Appl Pharmacol. 1967;10:481-500.
26. Foreman H, Vier M, Magee M. The metabolism of C14-labeled ethylenediaminetetraacetic acid in the rat. J Biol Chem. 1953;203:1045-53.
27. Srbrova JJT. Über die Resorption des Calciumnatriumsalzes der Äthylendiamintetraessigsäure bei der peroralen Verbreichung zur Therapie der Bleivergiftung. [On the resorption of the calcium disodium salt of EDTA after oral administration for treatment of lead poisoning.]. Archiv für Gewerbepathologie. 1957;15:572.
28. Spencer H. Studies of the effect of chelating agents in man. Ann NY Acad Sci. 1960;88(2):435-49.
29. AHFS Drug Information. Edetate Calcium Disodium: American Society of Health-System Pharmacists, Inc.; 2009. [cited 2013 08-10].

    Available: http://www.drugs.com/monograph/edetate-calcium-disodium.html - rb
30. Saquy PC, Maia-Campos G, Sousa-Neto MD, Guimaraes LF, P´ecora JD. Evaluation of chelating action of EDTA in association with Dakin's solution. Braz Dent J. 1994;5:65-70.
31. Martell AE. Chelation: Stability and selecitivty. Ann NY Acad Sci. 1960;88(2):284-92.
32. EC. European Union Risk Assessment Report: Edetic Acid (EDTA). Risk Assessment. European Chemicals Bureau. 2004;49.
33. Perry HM, Perry EF. Normal concentrations of some trace metals in human urine: changes produced by EDTA. J Clin Invest. 1959;38:1452-63.
34. Waters RS, Bryden NA, Patterson KY, Veillon C, Anderson RA. EDTA chelation effects on urinary losses of cadmium, calcium, chromium, cobalt, copper, lead, magnesium, and zinc. Biol Trace Elem Res. 2001;83(3):207-21.

35. Foreman H. Use of chelating agents in treatment of metal poisoning (with special emphasis on lead). Federation proceedings. 1961;20(10):191.

36. Allain P, Mauras Y, Premel-Cabic A, Islam S, Herve JP, Cledes J. Effects of an EDTA infusion on the urinary elimination of several elements in healthy subjects. Br J Clin Pharmacol. 1991;31(3):347-9.

37. Cory-Slechta DA, Weiss B, Cox C. Mobilization and redistribution of lead over the course of calcium disodium ethylenediamine tetraacetate chelation therapy. J Pharmacol Exp Ther. 1987;243(3):804-13.

38. Chen KH, Lin JL, Lin-Tan DT, Hsu HH, Hsu CW, Hsu KH, et al. Effect of Chelation Therapy on Progressive Diabetic Nephropathy in Patients With Type 2 Diabetes and High-Normal Body Lead Burdens. American Journal of Kidney Diseases. 2012;60(4):530-8.

39. Ernst E. Chelation therapy for coronary heart disease: An overview of all clinical investigations. American Heart Journal. 2000;140(1):139-41.

40. Lin JL, Lin-Tan DT, Hsu KH, Yu CC. Environmental lead exposure and progression of chronic renal diseases in patients without diabetes. New England Journal of Medicine. 2003;348(4):277-86.

41. Reuber MD, Bradley JE. Acute versenate nephrosis: Occurring as the result of treatment for lead intoxication. JAMA. 1960;174(3):263-9.

42. Moel DI, Kumar K. Reversible nephrotoxic reactions to a combined 2, 3-dimercapto-1-propanol and calcium disodium ethylenediaminetetraacetic acid regimen in asymptomatic children with elevated blood lead levels. Pediatrics. 1982;70(2):259-62.

43. Oser BL, Oser M, Spencer HC. Safety evaluation studies of calcium EDTA. Toxicol Appl Pharmacol. 1963;3//;5(2):142-62.

44. Bauer RO. Acute and subacute toxicity of ethylene diamine tetraacetic acid (EDTA) salts. Federation Proceedings. 1952;11:321.

45. Schwartz SL, Hayes JR, Ide RS, Johnson CB, Doolan PD. Studies of the nephrotoxicity of ethylenediaminetetraacetic acid. Biochemical pharmacology. 1966;15(3):377-89.

46. Schwartz SL, Johnson CB, Hayes JR, Doolan PD. Subcellular localization of ethylenediaminetetraacetate in the proximal tubular cell of the rat kidney. Biochemical Pharmacology. 1967;16(12):2413-9.

47. Ahrens FA, Aronson AL. A comparative study of the toxic effects of calcium and chromium chelates of ethylenediaminetetraacetate in the dog. Toxicol Appl Pharmacol. 1971;1//;18(1):10-25.

48. Araki S, Aono H, Murata K. Mobilisation of heavy metals into the urine by CaEDTA: relation to erythrocyte and plasma concentrations and exposure indicators. British journal of industrial medicine. 1986;43(9):636-41.

49. Kawamata K, Yoshimoto H, Momma J. Comparative toxicity studies of Na2EDTA and CaNa2EDTA in rats. Jpn J Pharmacol. 1980;30:234.

50. Sprague G. The effect of dietary administration of ethylenediaminetetraacetic acid upon the mineral content of mouse tissues. Kearney, Nebraska: Kearney State College; 1973.

51. Dagirmanjian R, Maynard EA, Hodge HC. The effects of Calcium Disodium Ethylenediamine Tetraacetate on Uranium poisoning in rats. J Pharmacol Exp Ther. 1956;117(1):20-8.

52. Reuber MD, Schmieler GC. Edetate Kidney lesions in rats. Arch Environ Health. 1962;5(5):430-6.

53. Foreman H, Finnegan C, Lushbaugh CC. Nephrotoxic hazard from uncontrolled edathamil calcium-disodium therapy. J Am Med Assoc. 1956;160(12):1042-6.

54. Reuber MD. Calcium disodium edetate nephrosis in female rats of varying ages. J Pathol. 1969;97(2):335-8.

55. Reuber MD. Hepatic lesions in young rats given calcium disodium edetate. Toxicol Appl Pharmacol. 1967;9//;11(2):321-6.

56. Reuber MD. Severe nephrosis in older male rats given calcium disodium edetate. Arch Environ Health. 1967;15(2):141-6.

57. Braide V. Total serotonin (5-hydroxytryptamine) concentrations in rat tissues after intravenous infusions of calcium EDTA. General Pharmacology: The Vascular System. 1975;6(1):59-61.

58. Egashira T, Sakai K, Sakurai M, Takayama F. Calcium disodium edetate enhances type A monoamine oxidase activity in monkey brain. Biol Trace Elem Res. 2003;94(3):203-11.

59. Braide V, Grill V, Delbello G. Calcium ethylenediaminetetraacetate toxicity in the rat: ultrastructural effects on skin collagen. Pharmacological research communications. 1988;20(2):133-46.

60. Aronson AL, Rogerson KM. Effect of calcium and chromium chelates of ethylenediaminetetraacetate on intestinal permeability and collagen metabolism in the rat. Toxicol Appl Pharmacol. 1972;4//;21(4):440-53.

61. Rosenblatt D, Aronson AL. Calcium ethylenediaminetetraacetate (CaEDTA) toxicity: time-and dose-response studies on intestinal DNA synthesis in the rat. Experimental and Molecular Pathology. 1978;28(2):202-14.

62. Krum JK. University of Massachusetts; 1948 (Thesis).

63. Yang SS, Chan MS. Summaries of toxicological data: Toxicity of EDTA. Food Cosmet Toxicol. 1964;2:763-7.

64. Chan MS. Some Toxicological and Physiological Studies of Ethylenediamine Tetraacetic Acid in the Albino Rat: University of Massachusetts; 1956.

65. Heimbach J, Rieth S, Mohamedshah F, Slesinski R, Samuel-Fernando P, Sheehan T, et al. Safety assessment of iron EDTA [sodium iron (Fe(3+)) ethylenediaminetetraacetic acid]: Summary of toxicological, fortification and exposure data. Food and chemical toxicology. An International Journal Published for the British Industrial Biological Research Association. 2000;38(1):99-111.

66. Gummow B, Botha C, Williams M. Chronic vanadium poisoning in calves and its treatment with calcium disodium ethylenediaminetetraacetate. Veterinary research communications. 2006;30(7):807-22.

67. Sroczynski J. The blood-forming system in the course of prolonged prophylactic administration of disodium-calcium versenate in experimental lead poisoning. Archivum immunologiae et therapiae experimentalis. 1966;14(5):670-7.

68. Heindorff K, Aurich O, Michaelis A, Rieger R. Genetic toxicology of ethylenediaminetetraacetic acid (EDTA). Mutation Research. 1983;115(2):149-73.

69. Gentile JM, Hyde K, Schubert J. Chromium genotoxicity as influenced by complexation and rate effects. Toxicol Lett. 1981;3//;7(6):439-48.

70. Corp MP. Calcium disodium versenate (edetate calcium disodium) injection [NDA008922]; 2013. [cited 2013 October 11th].

Available: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=74435a8a-28a9-4080-93d0-d9d5f10b325e - nlm34089-3.

71. Brownie CF, Brownie C, Noden D, Krook L, Haluska M, Aronson AL. Teratogenic effect of calcium edetate (CaEDTA) in rats and the protective effect of zinc. Toxicol Appl Pharmacol. 1986;3/15/;82(3):426-43.

72. Schardein JL, Sakowski R, Petrere J, Humphrey RR. Teratogenesis studies with EDTA and its salts in rats. Toxicol Appl Pharmacol. 1981;61(3):423-8.

73. Apgar J. Use of EDTA to produce zinc deficiency in the pregnant rat. The Journal of nutrition. 1977;107(4):539-45.

74. Abendroth VK. Ausgezeichnete Wirksamkeit der Natriumzitrat-EDTA-Kombinationstherapie bei Schwerer Bleivergiftung in der Schwangerschaft. Dtsch Ges Wissen. 1971;26:2130-1.

75. Angle CR, McIntire MS. Lead poisoning during pregnancy: Fetal tolerance of calcium disodium edetate. Am J Dis Child. 1964;108(4):436-9.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=513&id=30&aid=5317