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[1]

EDTA (ethylenediamine tetraacetic acid) and its salts are substituted diamines. HEDTA (hydroxyethyl ethylenediamine triacetic acid) and its trisodium salt are substituted amines. These ingredients function as chelating agents in cosmetic formulations. The typical concentration of use of EDTA is less than 2%, with the other salts in current use at even lower concentrations. The lowest dose reported to cause a toxic effect in animals was 750 mg/kg/day. These chelating agents are cytotoxic and weakly genotoxic, but not carcinogenic. Oral exposures to EDTA produced adverse reproductive and developmental effects in animals. Clinical tests reported no absorption of an EDTA salt through the skin. These ingredients are likely, however, to affect the passage of other chemicals into the skin because they will chelate calcium. Exposure to EDTA in most cosmetic formulations, therefore, would produce systemic exposure levels well below those seen to be toxic in oral dosing studies. Exposure to EDTA in cosmetic formulations that may be inhaled, however, was a concern. An exposure assessment done using conservative assumptions predicted that the maximum EDTA dose via inhalation of an aerosolized cosmetic formulation is below that shown to produce reproductive or developmental toxicity. Because of the potential to increase the penetration of other chemicals, formulators should continue to be aware of this when combining these ingredients with ingredients that previously have been determined to be safe, primarily because they were not significantly absorbed. Based on the available data, the Cosmetic Ingredient Review Expert Panel found that these ingredients are safe as used in cosmetic formulations.

INTRODUCTION

Ethylenediamine Tetraacetic Acid (EDTA) and its salts are substituted diamines; Hydroxyethyl Ethylenediamine Triacetic Acid (HEDTA) and its salt, Trisodium HEDTA, are substituted amines. These ingredients function as chelating agents in cosmetic formulations by combining with polyvalent metal cations in solution to form soluble ring structures. EDTA and its salts (known collectively as Edetates) have uses in foods, pharmaceutical products, and manufacturing, and are used to treat heavy metal poisoning and to reduce blood cholesterol.

CHEMISTRY

Definition and Structure

EDTA, HEDTA, and their salts conform to the formulas depicted in Figure 1 (Wenninger and McEwen 1997).

EDTA (CAS No. 60-00-4) is a substituted diamine. The food- and pharmaceutical-grade compound contains not less than 98.0% and not more than 100.5% of $C_{10}H_{16}N_2O_8$ (U.S. Pharmacopeial Convention, Inc. 1995). The cosmetic-grade material contains 99.0% to 102.0% EDTA (Nikitakis and McEwen 1990a).

Other names for EDTA include Edetic Acid; Edetic Acid (EDTA); Ethylene Diamine Tetra Acetic Acid; *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)Glycine]; Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)- (Wenninger and McEwen 1997); Ethylenediaminetetraacetic acid; Versenic Acid (Grant 1972); Edetate (Taylor 1988); (Ethylenedinitrilo)Tetraacetic Acid; Edathamil (Budavari 1989; Gennaro 1990; U.S. Pharmacopeial Convention, Inc. 1995); Ethylenedinitrilotetraacetic Acid; Ethylenebisiminodiacetic Acid (Lewis 1993); and 3,6-Diazooctanedioic Acid, 3,6-bis(Carboxymethyl)- [Registry of Toxic Effects of Chemical Substances (RTECS) 1997].

Calcium Disodium EDTA (CAS No. 62-33-9) is a substituted diamine (Wenninger and McEwen 1997). The food- and pharmaceutical-grade chemical 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 (Gennaro 1990; U.S. Pharmacopeial Convention, Inc. 1995).

Synonyms for Calcium Disodium EDTA are Calcium Disodium Ethylenediamine Tetraacetic Acid; Calcium Disodium Ethylenediaminetetraacetate; Edetate Calcium Disodium

Received 6 June 2002; accepted 6 June 2002.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Rebecca S. Lanigan and Torill A. Yamarik prepared this report. Address correspondence to F. Alan Andersen, Cosmetic Ingredient Review Director, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

International Journal of Toxicology, 21(Suppl. 2):95–142, 2002

1091-5818/02 $12.00 + .00
DOI: 10.1080/1091581029009652 2

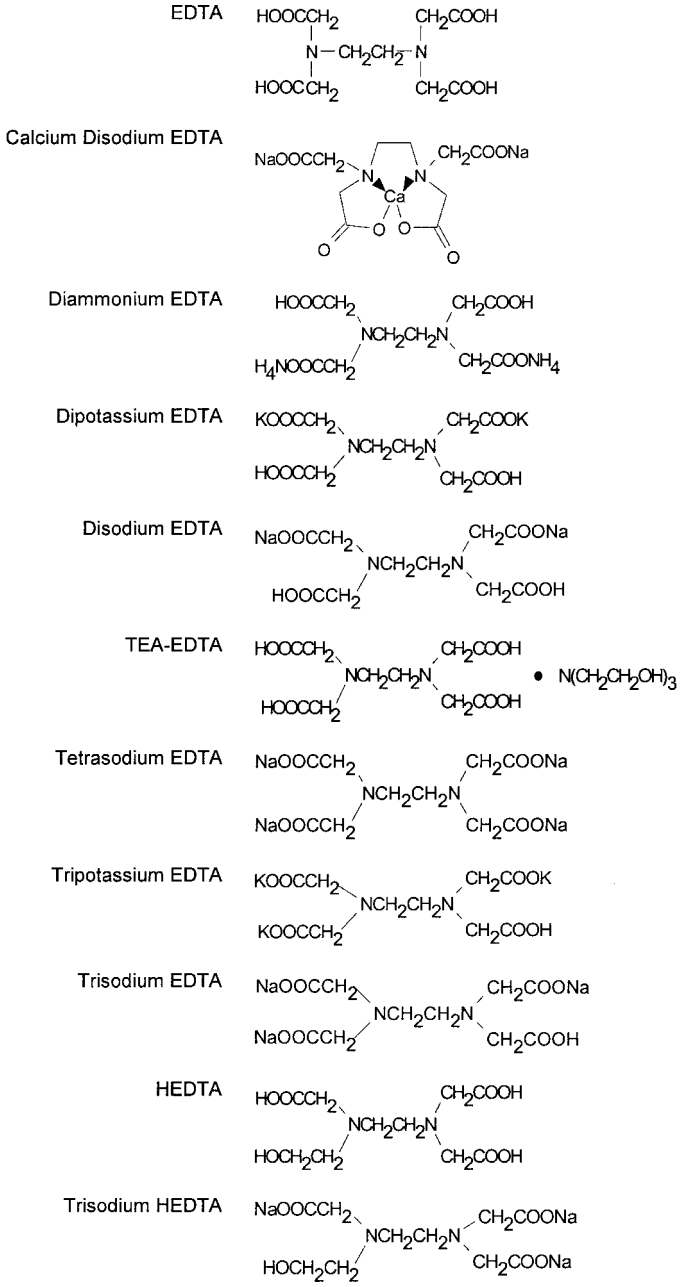

FIGURE 1
Chemical formulas for EDTA, HEDTA, and their salts.

(Wenninger and McEwen 1997); Calciate(2-), [[*N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-Glycinato]](4-)*N*,*N*′,*O*,*O*′, O^N,$O^{N'}$]-, Disodium, Hydrate, (OC-6-21)-; Disodium [(Ethylenedinitrilo)Tetraacetato] Calciate(2-) Hydrate (U.S. Pharmacopeial Convention, Inc. 1995); Edathamil Calcium Disodium; $CaNa_2EDTA$; Calcium Disodium (Ethylenedinitrilo)-Tetraacetate (Gosselin, Smith, and Hodge 1984); EDTA Calcium; Calcium Disodium Edetate; [(Ethylenedinitrilo)Tetraacetato]-Calciate(2-)Disodium; Ethylenediaminetetraacetic Acid Calcium Disodium Chelate; Calcium Disodium (Ethylenedinitrilo)-Tetraacetate; Edetic Acid Calcium Disodium Salt; Sodium Calciumedetate; Calcitetracemate Disodium; Calcium Disodium Versenate (Budavari 1989); Calcium Disodium Edathamil; and Calcium Sodium Edetate (Taylor 1988).

Diammonium EDTA (CAS No. 20824-56-0) is a substituted diamine that is also known as Diammonium Edetate; Diammonium *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)Glycine]; Edetate Diammonium; Diammonium Ethylenediaminetetraacetate; and Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-, Diammonium Salt (Wenninger and McEwen 1997).

Dipotassium EDTA (CAS No. 2001-94-7) is a substituted diamine. Synonyms for this ingredient are Dipotassium Edetate Dihydrate; *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl) Glycine], Dipotassium Salt; and Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-, Dipotassium Salt (Wenninger and McEwen 1997).

Disodium EDTA (CAS No. 139-33-3) is a substituted diamine (Wenninger and McEwen 1997). When dried, the cosmetic-grade material contains not less than 99.0% of Disodium EDTA (Yakugi Nippo Ltd. 1979); it contains 76.0% to 77.5% EDTA (Nikitakis and McEwen 1990a). The pharmaceutical-grade material contains not less than 99.0% and not more than 101.0% of $C_{10}H_{14}N_2Na_2O_8$, calculated on a dried basis (U.S. Pharmacopeial Convention, Inc. 1995).

Synonyms for Disodium EDTA include Disodium Edetate; Disodium *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)Glycine]; Disodium Ethylenediaminetetraacetate; Edetate Disodium; Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-, Disodium Salt (Wenninger and McEwen 1997); Sodium Versenate; Disodium (Ethylenedinitrilo) Tetraacetate Dihydrate (Gennaro 1990); (Ethylenedinitrilo)-Tetraacetic Acid Disodium Salt; Ethylenediaminetetraacetic Acid Disodium Salt; Ethylenebis (Iminodiacetic Acid) Disodium Salt; Disodium Edathamil; EDTA Disodium; Tetracemate Disodium; Disodium Ethylenediaminetetraacetate; Endrate Disodium; Versene Disodium Salt; Edathamil Disodium (Budavari 1989); and Sequestrene (Sax 1979).

TEA-EDTA (CAS No. 60544-70-9) is a triethanolamine (TEA) salt of EDTA (q.v.). Synonyms for this ingredient are Triethanolamine EDTA and Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-Compound with 2,2′,2″-Nitrilotris[Ethanol] (Wenninger and McEwen 1997).

Tetrasodium EDTA (CAS No. 64-02-8) is a substituted amine. The cosmetic-grade material contains a minimum of 99.0% Tetrasodium EDTA as $Na_4EDTA \cdot 2H_2O$ (Nikitakis and McEwen 1990b; Wenninger and McEwen 1997).

Tetrasodium EDTA is also known as Edetate Sodium; *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)Glycine], Tetrasodium Salt; Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-, Tetrasodium Salt; Sodium Edetate; Tetrasodium Edetate; Tetrasodium Edetate Dihydrate; Tetrasodium Ethylene Diamine Tetraacetate (Wenninger and McEwen 1997); Ethylenediaminetetraacetic Acid Tetrasodium Salt; Ethylenebis(Iminodiacetatic

Acid) Tetrasodium Salt; Tetrasodium Ethylenebis (Iminodiacetate); EDTA Disodium; Edetic Acid Tetrasodium Salt; Tetracemate Tetrasodium; Tetracemin; Endrate Tetrasodium (Budavari 1989); Edathamil Tetrasodium; and Tetrasodium Ethylenediamine-*N*,*N*,*N*′,*N*′-Tetraacetate (Hazardous Substances Data Base [HSDB] 1998).

Tripotassium EDTA (CAS No. 17572-97-3) is a substituted diamine that is also known as Edetate Tripotassium; *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)Glycine], Tripotassium Salt; and Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-, Tripotassium Salt (Wenninger and McEwen 1997).

Trisodium EDTA (CAS No. 150-38-9) is a substituted amine. The cosmetic-grade material contains a minimum of 98% of Trisodium EDTA (Nikitakis and McEwen 1990a; Wenninger and McEwen 1997).

Trisodium EDTA is also known as Trisodium EDTA, Monohydrate (Nikitakis and McEwen 1990a); Edetate Trisodium; *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl) Glycine], Trisodium Salt; Glycine, *N*,*N*′-1,2-Ethanediylbis[*N*-(Carboxymethyl)-, Trisodium Salt; Trisodium Edetate; Trisodium Ethylenediamine Tetraacetate; Trisodium Hydrogen Ethylene Diaminetetraacetate (Wenninger and McEwen 1997); Trisodium Ethylenediaminetetraacetate; Ethylenediaminetetraacetic Acid Trisodium Salt; and Edetic Acid Trisodium Salt (Budavari 1989).

HEDTA (CAS No. 150-39-0) is a substituted amine. Synonyms for this compound are *N*-[2-[Bis(Carboxymethyl) Amino] Ethyl]-*N*-(2-Hydroxyethyl)Glycine; Hydroxyethyl Ethylenediamine Triacetic Acid; Glycine, *N*-[2-[Bis(Carboxymethyl) Amino]Ethyl]-*N*-(2-Hydroxyethyl)- (Wenninger and McEwen 1997); Hydroxyethylethylenediaminetriacetic Acid (Lewis 1993); HEEDTA (Hart 1984; HSDB 1998; RTECS 1997); (*N*-Hydroxyethylethylenedinitrilo)Triacetic Acid; Oxyethylethylenediaminetriacetic Acid; (2-Hydroxyethyl) Ethylenediaminetriacetic Acid; *N*-(Beta-Hydroxyethylethylenediamine)-*N*,*N*′,*N*′-Triacetic Acid; *N*-(Hydroxyethyl) Ethylenediaminetriacetic Acid; and *N*-(2-Hydroxyethyl)Ethylenediamine-*N*,*N*′,*N*′-Triacetic Acid (HSDB 1998).

Trisodium HEDTA (CAS No. 139-89-9) is a substituted amine that is also known as *N*-[2-Bis(Carboxymethyl)Amino]-Ethyl]-*N*-(2-Hydroxyethyl)Glycine, Trisodium Salt; Glycine, *N*-[2-Bis(Carboxymethyl)Amino]Ethyl]- *N*-(2-Hydroxyethyl)-, Trisodium Salt; Trisodium Hydroxyethyl Ethylenediaminetriacetate; Trisodium Hydroxyethyl Ethylenediamine Triacetate (Dihydrate); and Trisodium Hydroxyethyl Ethylenediamine Triacetate Solution (Wenninger and McEwen 1997).

Chemical and Physical Properties

EDTA is a white, odorless, nonhygroscopic crystalline powder. It decomposes over a melting range of 234°C to 250°C. It contains a maximum of 50 ppm of heavy metals (as Pb), and has a 0.5% maximum residue on ignition (Nikitakis and McEwen 1990a). The molecular weight of this compound is ~292.24 Da (Budavari 1989; Aldrich Chemical Company 1992; U.S. Pharmacopeial Convention 1995). EDTA is slightly soluble in water, soluble in solutions of alkali hydroxides, and is insoluble in common organic solvents (Gennaro 1990; Lewis 1993).

Calcium Disodium EDTA is a white to off-white, crystalline or granular powder with a faint, saline-like taste that is odorless and slightly hygroscopic. It is soluble in water such that a 0.1 M solution (pH = 7) can be prepared at 30°C; 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 (anhydrous) is 374.27 to 410.3 Da and the melting point is >300°C (Budavari 1989; Gennaro 1990; Aldrich Chemical Company 1992; U.S. Pharmacopeial Convention 1995; National Academy of Sciences [NAS] 1996).

Diammonium EDTA has a molecular weight of 326.31 Da and decomposes at 218°C (Aldrich Chemical Company 1992).

Dipotassium EDTA has a molecular weight of 404.47 Da and decomposes at 272°C (Aldrich Chemical Company 1992).

Disodium EDTA is a water-soluble, almost odorless, white crystal or crystalline powder with a molecular weight of 336.21 to 372.24 Da. The pH of a 1:100 solution is 4.3 to 4.7 and the pH of a 1:20 solution is 4.0 to 6.0. Disodium EDTA decomposes at 252°C. The melting point of Disodium EDTA is 240°C, the flash point is >100°C, and it is soluble in water (~100 g/l) at 20°C (Yakuji Nippo, Ltd. 1979; Budavari 1989; Gennaro 1990; NAS 1996; BASF 1996a).

Tetrasodium EDTA has a molecular weight of ~380.2 Da and a melting point of >300°C. It is a powder with an apparent density of 6.9 lb/gallon and is very soluble in water (103 g/100 ml). It is less soluble in alcohol than the potassium salt (Budavari 1989; Aldrich Chemical Company 1992). The pH of a 1% Tetrasodium EDTA solution at 25°C is 11 to 12 (J. Lowenstein and Sons, Inc. 1996).

Tripotassium EDTA has a molecular weight of 442.56 Da and decomposes at 182°C (Aldrich Chemical Company 1992).

Trisodium EDTA is a white crystalline powder with a molecular weight of ~358.2 Da and a melting point of >300°C. It is more soluble in water than Disodium EDTA or the corresponding free acid, and is insoluble in organic solvents. The pH of a 1% aqueous solution is 9.3 to 12 (Budavari 1989; Nikitakis and McEwen 1990a; Aldrich Chemical Company 1992).

Analytic Methods

Disodium EDTA has been determined in ophthalmic and contact lens care solutions using reversed-phase high-performance liquid chromatography (Hall and Takahashi 1988) and differential pulse polarography (Carlson and Habeger 1980). Hamano et al. (1985) used column chromatography to perform a separative determination of Disodium EDTA and EDTA-metals in foods.

Ultraviolet Absorbance

Tetrasodium EDTA (4 mM in 20 mM HEPES buffer; pH = 7.4) had an absorption peak at 280 nm. When the salt was dissolved in water, the absorption changed as a function of pH. A

concentration of 10 mM Tetrasodium EDTA was titrated with HCl. At pH 11, the absorption intensity (A_{240}) was 2.8; at pH 8, the A_{240} was 2.45; at pH 4, the A_{240} was 0.2; and at pH 2, the A_{240} was 0.55. The further addition of HCl precipitated EDTA as free acid (Müller and Haeberli 1994).

Lee and Pirt (1979) reported that 1 g/l EDTA decomposed (to 0.4%) when exposed to sunlight for 53 h; the ultraviolet (UV) absorption peak occurred at ~210 nm. Culture media containing 0.25 g/l (0.86 mmol) EDTA also decomposed. Fresh media had three absorption peaks at 192, 202, and 250 nm, whereas exposed media had no band at 250 nm.

Reactivity

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 (Budavari 1989; Gennaro 1990; Lewis 1993). Chelating agents such as EDTA are anionic. EDTA forms a tetranegative anion, and is 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 (Hart 1984). 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 (Saquy et al. 1994).

Generally, EDTA is stable as a solid and in aqueous solution. Only strong oxidizing agents can attack it chemically. The stability of EDTA-metal chelates increases according to the order:

$$Na^{+} < Ca^{2+} < Fe^{2+} < Co^{2+} < Zn^{2+} < Cu^{2+} < Pb^{2+} < Fe^{3+}$$

The chelates are water-soluble, but are not ionized in aqueous solution (Heindorff et al. 1983). The affinity of HEDTA for divalent metal ions is similar to that of EDTA in the acid pH range, but metal chelates of HEDTA are much more susceptible to metal hydrolysis and other interferences in the alkaline pH range. In general, the difference in metal ion affinity of EDTA over HEDTA is greater for less basic ions (Chaberek and Martell 1955).

EDTA can enhance the effects of natural antioxidants (e.g., ascorbic acid) in vegetable oils by eliminating or deactivating the effects of metal-catalyzing autoxidation, but it has little to no effect in animal fats (Swern 1979).

EDTA is less stable than its salts and tends to decarboxylate when heated to 150°C. It is stable when stored and when boiled in aqueous solution (Budavari 1989). Calcium Disodium EDTA is stable in air (Gennaro 1990). Disodium EDTA has some characteristics of weak acids; it displaces carbon dioxide from carbonates and reacts with metals to form hydrogen (Budavari 1989).

Müller and Haeberli (1994) reported that EDTA can form supramolecular aggregates, the formation of which was a function of pH. The investigators based their conclusion on results of UV spectroscopy, gel permeation chromatography, dialysis, and cryoelectron microscopy. At pH 4, EDTA had the maximum number of charges (four negative from carboxylates, two positive from the imino moieties), which gave the molecule maximum solubility and minimum UV absorption. The removal of protons induced increases of UV absorption, which was interpreted as the formation of aggregates. At basic pH, the molecule was strongly polarized, with each edge having two negative charges separated by an uncharged and apolar region. The aggregates were present at pHs in the physiological range. The molecular weights of the aggregates were 10,000 to 15,000 and 8000 to 14,000 Da, as determined using gel chromatography and dialysis, respectively. Cryoelectron microscopy produced evidence of structures of 2- to 4-nm diameter.

Method of Manufacture

All of the industrially used methods of manufacture of EDTA and its salts involve the addition of formaldehyde and hydrogen cyanide or an alkali metal cyanide to an aqueous solution of EDTA. The salts, or edetates, are then formed by hydrolysis (HSDB 1998). EDTA can also be formed by heating tetrahydroxyethylethylenediamine with sodium or potassium hydroxide using a cadmium oxide catalyst (Lewis 1993).

EDTA and Trisodium EDTA were formed when ethylenediamine was condensed with sodium monochloroacetate in the presence of sodium carbonate. An aqueous solution of the reactants was heated to 90°C for 10 hours, then was cooled and acidified with hydrochloric acid, whereupon the acid precipitated (Gennaro 1990).

Calcium Disodium EDTA was produced by boiling an aqueous solution of Disodium EDTA with slightly more than an equimolar quantity of calcium carbonate until carbon dioxide was no longer evolved. The resulting solution was filtered while hot and crystallized (Gennaro 1990).

Disodium EDTA was prepared by dissolving EDTA into a hot solution that contained two equivalents of sodium hydroxide. The solution was then allowed to crystallize (Gennaro 1990).

Tetrasodium EDTA was formed by reacting a basic solution of ethylenediamine with formaldehyde and sodium or hydrogen cyanide, followed by hydrolysis to form the salt (Lewis 1993). It can also be produced by using excess sodium hydroxide in the reaction. The intermediate ethylenediaminetetracetonitrile can be isolated and hydrolyzed to ammonium EDTA in a separate step (HSDB 1998). It was supplied either in anhydrous form or hydrated with two molecules of water per molecule of the salt (Lewis 1993).

Trisodium EDTA was formed by heating tetrahydroxyethylethylenediamine with sodium or potassium hydroxide with calcium oxide catalyst (Lewis 1993).

Impurities

In a submission to the Food and Drug Administration (FDA), the Dow Chemical Company (1987) reported that mixtures

comprised mainly of pentasodium EDTA or Trisodium EDTA can contain "very small amounts" (not quantified) of nitrilotriacetate (NTA). NTA is listed as a potential carcinogen by the National Toxicology Program (NTP).

The submission provided the following analysis: "Although large dietary doses of [NTA] have caused urinary tumors in laboratory animals, there is little likelihood that it could cause cancer in humans, especially at subtoxic doses . . . It is evident from a review of the animal data that extrapolation from data from rats to man is inappropriate since NTA is readily absorbed from the [GI] tract of the rat and poorly absorbed from the [GI] tract of man. The primary excretory route for NTA in the rat is via the urine. At comparable dose levels the 72 hour urinary excretions of NTA given as a percent of administered dose are: rat, 95%; dog, 69%; rabbit, 23%; monkey, 14%. Man has been reported to excrete NTA much like the rabbit and monkey, approximately 90% of the oral dose being excreted in 72 hours, 12% of which is excreted in the urine and 77% in the feces. NTA is not significantly biotransformed by mammals. In the rat less than 1% was expired as CO_2 in 72 hours" (Dow Chemical Company 1987).

Fregert and Gruvberger (1980) reported that Tetrasodium EDTA could contain an unspecified amount of the starting material, formaldehyde.

Disodium EDTA is not expected to contain preservatives, antioxidants, or sequestering agents for the purposes of stabilization. Disodium EDTA is not expected to contain pesticides, 1,4-dioxane, free ethylene oxide, monochloroacetic acid, sulfite, organic solvents, nitrosamines, or other substances. The maximum concentration of heavy metals and formaldehyde is 10 ppm and 100 ppm, respectively (BASF 1996b).

USE

Cosmetic

EDTA, HEDTA, and their salts function as chelating agents in cosmetics (Wenninger and McEwen 1997). EDTA, Dipotassium EDTA, Disodium EDTA, Tetrasodium EDTA, Tripotassium EDTA, Trisodium EDTA, HEDTA, and Trisodium HEDTA were reported to FDA as used in a total of 4168 cosmetic formulations, whereas Calcium Disodium EDTA, Diammonium EDTA, and TEA-EDTA were not reported as used in cosmetics (FDA 1998a). Tables 1 through 8 present the use of these ingredients as a function of product type and give current concentration of use information. The total formulation per category number in column 1 is provided as a point of comparison for the number of formulations that contain an ingredient. For example, in Table 1, only 1 out of 200 bubble baths on the market are reported to contain EDTA, whereas 663 of the 1572 hair dyes and colors contain EDTA. In some cases, a concentration of use of EDTA was provided by industry in a product category for which no uses had been reported to FDA. There is no way of knowing the number of uses that corresponds to that concentration, so the total formulation per category number in column one is omitted.

Historical data (data submitted to the FDA in 1984) indicated that EDTA and Tetrasodium EDTA were used typically at concentrations up to 1%, but one formulation each contained concentrations of 25%. Disodium EDTA and Trisodium were reported used at concentrations of $\leq$5%, but the typical concentrations used were $\leq$1% and $\leq$0.1%, respectively. Trisodium HEDTA was used at a concentration of 10% in one formulation, but was more often used at concentrations of $\leq$1%. Dipotassium EDTA and Tripotassium EDTA were used at unknown concentrations, and Calcium Disodium EDTA, Diammonium EDTA, TEA-EDTA, and HEDTA were not reported used (FDA 1984).

In March 1999, the Cosmetic, Toiletry, and Fragrance Association (CTFA) asked companies that reported using EDTA or HEDTA chelators if they used them in spray products and, if so, what was the maximum concentration used. The companies that responded indicated the following maximum concentrations in spray products: Disodium EDTA 0.05% to 0.1%, Trisodium EDTA 0.012%, and Tetrasodium EDTA 0.2%. Other companies that responded indicated that they did not use these chelators in spray products (CTFA 1999).

Noncosmetic

The use of EDTA and its salts as stabilizing and chelating agents has been described as "ubiquitous" (Rietschel and Fowler 1995). Originally, EDTA and the Edetates were developed to counteract the effects of hard water and heavy metal ions on dyestuffs used in textile manufacture. Currently, these ingredients are used in a wide number of applications. EDTA, HEDTA, and their salts maintain clarity, protect fragrance components, stabilize polymeric thickeners and color additives, prevent rancidity, and increase preservative effectiveness, among other effects (Hart 1984).

In foods, EDTA is an antioxidant (NTP 1998); Disodium EDTA is a preservative, sequestrant, and stabilizer; and Calcium Disodium EDTA is a preservative, sequestrant (NAS 1996), flavoring agent, and color retention agent (Budavari 1989; FDA 1998b). Disodium EDTA is permitted in the feed and drinking water of animals and/or for the treatment of food-producing animals (Sax 1979). EDTA controls corrosion of canned carbonated beverages, suppresses off-flavor in whole milk, enhances foaming properties of reconstituted skim milk, and retards nitric oxide–hemoglobin formation in cured meats (HSDB 1998). It has been estimated that daily human consumption of the EDTA salts are 1.5×10^{-4}, 2.3×10^{-1}, and 3.4×10^{-2} mg/kg for Tetrasodium EDTA, Calcium Disodium EDTA, and Disodium EDTA, respectively (FDA 1998b).

EDTA and the Edetates prevent discoloration due to trace metals in antibiotics, antihistamines, and local anesthetics. They are used to stabilize solutions of ascorbic acid (vitamin C), hydrogen peroxide, formaldehyde, folic acid, and hyaluronidase and are used to increase antibacterial activity of components of ophthalmic and contact lens care solutions (Hall and Takahashi 1988; Rietschel and Fowler 1995). EDTA is a clarifying agent that prevents the formation of calcium, magnesium, and iron

TABLE 1
Use of EDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Other baby products	—	0.03%
Bubble baths (200)	1	—
Other bath preparations (159)	2	—
Eyeliner (514)	1	—
Eye lotion (18)	1	—
Eye makeup remover (84)	2	0.03%–0.05%
Mascara (167)	1	—
Colognes and toilet waters (656)	1	—
Other fragrance preparations (148)	1	—
Hair conditioners (636)	3	—
Hair straighteners (63)	1	—
Permanent waves (192)	4	0.05%
Rinses—noncoloring (40)	1	—
Shampoos—noncoloring (860)	58	0.2%–0.3%
Tonics, dressings, and other hair-grooming aids (549)	3	0.05%–0.1%
Wave sets (55)	2	0.05%
Hair dyes and colors (1572)	663	0.2%–2%
Hair tints (54)	20	0.05%
Hair shampoos—coloring (24)	1	—
Hair lighteners with color (6)	4	2%
Hair bleaches (113)	34	1%
Other hair-coloring preparations (59)	3	0.02%–2%
Face powders (250)	2	0.1%
Foundations (287)	2	0.1%–0.5%
Other makeup preparations (135)	1	—
Nail basecoats and undercoats (48)	2	—
Nail creams and lotions (17)	1	—
Bath soaps and detergents (385)	7	0.05%–0.3%
Deodorants—underarm (250)	3	0.1%–0.5%
Other personal cleanliness products (291)	9	0.2%
Skin cleansing preparations (653)	17	0.05%
Face and neck preparations—not shaving (263)	7	—
Body and hand preparations—not shaving (796)	8	0.05%–0.5%
Moisturizing preparations (769)	15	0.1%
Night preparations (188)	5	—
Paste masks—mud packs (255)	6	—
Skin fresheners (184)	3	—
Other skin care preparations (692)	16	0.1%
Suntan gels, creams, and liquids (136)	9	0.05%
Other suntan preparations (38)	3	—
Face treatment	—	0.03%
Skin protectant	—	0.1%
Total uses/concentration ranges of EDTA	**923**	**0.02%–2%**

TABLE 2
Use of Dipotassium EDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing Dipotassium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Eye makeup remover (84)	1	—
Skin cleansing preparations (653)	3	0.05%–0.09%
Face and neck preparations—not shaving (796)	3	0.09%
Body and hand preparations—not shaving (796)	3	0.09%
Moisturizing preparations (769)	1	—
Night preparations (188)	3	—
Paste masks—mud packs (255)	1	—
Other skin care preparations (692)	1	—
Suntan gels, creams, and liquids (136)	5	—
Total uses/concentration ranges of Dipotassium EDTA	**21**	**0.05%–0.09%**

TABLE 3
Use of Disodium EDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing Disodium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Baby shampoos (21)	1	—
Baby lotions, oils, powders, and creams (53)	1	—
Other baby products (29)	3	—
Bath oils, tablets, and salts (124)	3	0.1%–1%
Bubble baths (200)	24	0.1%
Other bath preparations (159)	50	0.2%
Eyebrow pencil	—	0.2%
Eyeliner	—	0.6%
Eye shadow	—	0.2%
Eye lotion (18)	2	0.1%–0.2%
Eye makeup remover (84)	10	0.2%–0.3%
Mascara (167)	2	0.1%–0.2%
Other eye makeup preparations (120)	19	0.1%
Colognes and toilet waters (656)	10	0.2%
Perfumes (195)	1	0.05%
Other fragrance preparations (148)	22	0.1%
Hair conditioners (636)	95	0.005%–0.25%
Hair sprays—aerosol fixatives	—	0.05%
Hair straighteners	—	0.2%
Permanent waves (192)	9	0.03%–0.5%
Rinses—noncoloring (40)	5	—
Shampoos—noncoloring (860)	131	0.2%–0.8%
Tonics, dressings, and other hair-grooming aids (549)	36	0.09%–0.2%
Wave sets (55)	3	—
Other hair preparations (276)	23	—
Hair dyes and colors (1572)	33	0.2%–0.4%

(Continued on next page)

TABLE 3
Use of Disodium EDTA in cosmetic formulations *(Continued)*

Product category (total formulations in category) (FDA 1998a)	Formulations containing Disodium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Hair tints (54)	1	—
Hair shampoos—coloring (24)	9	—
Hair bleaches (113)	27	—
Other hair-coloring preparations (59)	6	0.05%
Face powders	—	0.02%–0.05%
Foundations (287)	13	0.02%–0.5%
Lipstick	—	0.05%
Makeup bases (132)	2	—
Rouges (12)	1	—
Makeup fixatives (11)	1	0.02%
Other makeup preparations (135)	3	0.05%–0.2%
Nail creams and lotions	—	0.02%
Nail polish and enamel removers (34)	1	—
Other manicuring preparations	—	0.001%
Dentifrices	—	0.1%
Bath soaps and detergents (385)	30	0.06%–0.2%
Deodorants—underarm (250)	2	0.2%
Other personal cleanliness products (291)	23	0.1%–0.3%
Aftershave lotion (216)	22	0.01%–0.5%
Preshave lotions	—	0.1%
Shaving cream (139)	5	—
Shaving soap (<4)	1	—
Other shaving preparation products (60)	6	0.2%
Skin cleansing preparations (653)	73	0.05%–0.5%
Depilatories (28)	1	—
Face and neck preparations—not shaving (263)	32	0.05%–0.3%
Body and hand preparations—not shaving (796)	88	0.05%–0.6%
Foot powders and sprays (35)	5	0.05%
Moisturizing preparations (769)	132	0.05%–0.5%
Night preparations (188)	27	0.05%
Paste masks—mud packs (255)	28	0.02%–0.5%
Skin fresheners (184)	27	0.05%–0.5%
Other skin care preparations (692)	67	0.01%–0.2%
Suntan gels, creams, and liquids (136)	18	0.1%–0.5%
Indoor tanning preparations (62)	16	0.02%–0.1%
Other suntan preparations (38)	15	—
Astringents	—	0.1%
Body oils	—	0.05%
Face treatment	—	0.2%
Gels	—	0.1%
Skin protectants	—	0.2%
Soap bars	—	0.2%
Total uses/concentration ranges of Disodium EDTA	**1165**	**0.001%–0.8%**

TABLE 4
Use of Tetrasodium EDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing Tetrasodium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Baby shampoos (21)	7	0.05%–0.2%
Baby lotions, oils, powders, and creams (53)	1	0.1%
Other baby products (29)	6	0.3%
Bath oils, tablets, and salts (124)	5	—
Bubble baths (200)	49	0.01%–0.1%
Bath capsules (<4)	1	—
Other bath preparations (159)	50	0.05%–0.1%
Eyebrow pencil	—	0.1%
Eyeliner (514)	1	0.2%
Eye shadow (506)	5	0.5%
Eye lotion (18)	2	0.2%–0.3%
Eye makeup remover (84)	7	0.004%–0.2%
Mascara (167)	7	0.05%–0.2%
Other eye makeup preparations (120)	2	0.05%–0.2%
Colognes and toilet waters (656)	6	0.04%
Other fragrance preparations (148)	5	—
Hair conditioners (636)	48	0.02%–0.1%
Hair sprays—aerosol fixatives	—	0.08%
Hair straighteners (63)	2	0.2%
Permanent waves (192)	32	0.2%
Rinses—noncoloring (40)	4	—
Shampoos—noncoloring (860)	254	0.05%–1.3%
Tonics, dressings, and other hair-grooming aids (549)	31	0.04%–0.1%
Wave sets (55)	3	—
Other hair preparations—noncoloring (276)	33	0.05%–0.2%
Hair dyes and colors (1572)	215	0.3%–0.4%
Hair shampoos—coloring (24)	2	—
Hair lighteners with color (6)	1	—
Hair bleaches (113)	5	—
Other hair-coloring preparations (59)	2	—
Blushers (238)	12	0.5%
Face powders (250)	1	0.04%
Foundations (287)	15	0.01%–0.09%
Lipstick (790)	1	—
Makeup bases (132)	1	—
Makeup fixatives	—	0.01%
Other makeup preparations (135)	3	0.1%
Nail creams and lotions (17)	1	—
Cuticle softeners (19)	3	—
Other manicuring preparations	—	0.2%
Dentifrices	—	0.02%
Other oral hygiene products	—	0.009%
Bath soaps and detergents (385)	113	0.009%–0.5%
Deodorants—underarm (250)	9	0.04%–0.3%
Other personal cleanliness products (291)	46	0.09%–0.2%
Aftershave lotion (216)	15	0.1%–0.3%

(Continued on next page)

TABLE 4
Use of Tetrasodium EDTA in cosmetic formulations *(Continued)*

Product category (total formulations in category) (FDA 1998a)	Formulations containing Tetrasodium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Shaving cream (139)	5	0.08%–0.5%
Other shaving preparation products (60)	1	—
Skin cleansing preparations (653)	71	0.02%–0.5%
Face and neck preparations—not shaving (263)	17	0.04%–0.3%
Body and hand preparations—not shaving (796)	66	0.04%–0.1%
Foot powders and sprays (35)	4	—
Moisturizing preparations (769)	43	0.05%–0.1%
Night preparations (188)	9	—
Paste masks—mud packs (255)	7	0.02%–0.1%
Skin fresheners (184)	12	0.04%–0.09%
Other skin care preparations (692)	33	0.005%–0.2%
Suntan gels, creams, and liquids (136)	8	0.2%
Indoor tanning preparations (62)	2	—
Other suntan preparations (38)	1	0.08%
Astringents	—	0.09%
Face treatment	—	0.2%
Gels	—	0.04%
Skin protectants	—	0.04%
Soap bars	—	0.05%
Total uses/concentration ranges of Tetrasodium EDTA	**1285**	**0.004%–1.3%**

soaps that can cause turbidity (Lewis 1993; Rietschel and Fowler 1995).

EDTA and the Edetates are used in the production or processing of rubber and textiles (Grant 1972). 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 color film processing; and in analytic chemistry and spectrophotometric titrations (Lewis 1993; HSDB 1998).

Clinically, EDTA and Calcium Disodium EDTA are used intramuscularly (IM) or intravenously (IV) for the diagnosis and treatment of heavy metal poisoning, including exposure to lead, vanadium, and cadmium (Done 1979; Cantilena and Klaassen 1980; Taylor 1988; Rietschel and Fowler 1995). EDTA and the Edetates are used as anticoagulants in blood and to treat calcinosis (Lewis 1993), and Disodium EDTA is used occasionally to terminate the effects of injected calcium, to antagonize digitalis toxicity, or to suppress tachyarrhythmias (Gennaro 1990). Calcium Disodium EDTA is also added to pharmaceuticals to prevent calcium depletion in the body (Budavari 1989). EDTA has been used to treat alkali, particularly lime, burns of the cornea (Praus, Brettschneider, and Krejčí 1976). [^{51}Cr]-EDTA has been used since 1966 as a radiotracer for the assessment of glomerular filtration rate (Monteiro et al. 1994). Chelation therapy using EDTA has been used since 1955 to treat atherosclerotic cardiovascular disease, but its efficacy has been disputed in recent years (Brouwers 1984; Magee 1985; Grier and Meyers 1993). Disodium EDTA is also used at concentrations of 10% to 15% in dentistry to eliminate the smear layer before applying dentin adhesives and in root canal therapy for chemomechanical enlargement of canals (Segura et al. 1996).

TABLE 5
Use of Tripotassium EDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing Tripotassium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Eye makeup remover (84)	1	—
Total uses/concentration ranges of Tripotassium EDTA	**1**	—

TABLE 6
Use of Trisodium EDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing Trisodium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Other baby products (29)	1	—
Bath oils, tablets, and salts (124)	1	0.01%
Bubble baths (200)	2	0.05%–0.4%
Bath capsules	—	0.1%
Other bath preparations (159)	4	—
Eyebrow pencil (91)	1	0.05%–0.2%
Eyeliner (514)	16	0.05%–0.2%
Eye shadow (506)	59	0.05%–0.1%
Eye lotion (18)	4	0.05%–0.1%
Eye makeup remover (84)	9	0.05%–0.1%
Mascara (167)	24	0.1%–0.2%
Other eye makeup preparations (120)	15	0.3%
Colognes and toilet waters (656)	2	0.00001%
Perfumes	—	0.00001%
Powders (247)	2	—
Other fragrance preparations (148)	7	—
Hair conditioners (636)	8	0.03%–0.1%
Hair sprays—aerosol fixatives (261)	2	0.01%
Shampoos—noncoloring (860)	12	0.2%–0.4%
Tonics, dressings, and other hair-grooming aids (549)	3	0.01%–0.2%
Wave sets (55)	3	—
Other hair preparations (276)	1	—
Hair dyes and colors (1572)	17	0.5%
Other hair-coloring preparations (59)	1	0.1%
Blushers (238)	4	0.1%–0.2%
Face powders (250)	9	0.07%–0.2%
Foundations (287)	50	0.002%–0.2%
Lipstick (790)	3	—
Makeup bases (132)	19	0.05%–0.3%
Rouges (12)	1	—
Makeup fixatives (11)	1	0.05%
Other makeup preparations (135)	7	0.05%
Cuticle softeners (19)	5	0.2%
Nail creams and lotions (17)	3	0.1%
Other manicuring preparations (61)	2	—
Mouthwashes and breath fresheners (49)	1	—
Bath soaps and detergents (385)	10	0.1%–2%
Deodorants—underarm (250)	1	0.2%
Other personal cleanliness products (291)	6	—
Aftershave lotion (216)	8	0.01%–0.05%
Shaving cream (139)	3	0.05%–0.2%
Other shaving preparation products (60)	4	0.1%
Skin cleansing preparations (653)	44	0.05%–0.2%
Face and neck preparations—not shaving (263)	10	0.02%–0.2%
Body and hand preparations—not shaving (796)	53	0.03%–0.2%
Foot powders and sprays (35)	2	—

(Continued on next page)

TABLE 6
Use of Trisodium EDTA in cosmetic formulations *(Continued)*

Product category (total formulations in category) (FDA 1998a)	Formulations containing Trisodium EDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Moisturizing preparations (769)	74	0.1%–0.2%
Night preparations (188)	19	0.05%–0.1%
Paste masks—mud packs (255)	12	0.05%–0.2%
Skin fresheners (184)	9	0.05%–0.2%
Other skin care preparations (692)	51	0.1%
Suntan gels, creams, and liquids (136)	1	0.02%–0.2%
Indoor tanning preparations (62)	8	0.05%–0.5%
Other suntan preparations (38)	2	—
Total uses/concentration range of Trisodium EDTA	**616**	**0.00001%–2%**

TABLE 7
Use of HEDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing HEDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Permanent waves (192)	1	—
Total uses/concentration range of HEDTA	**1**	—

TABLE 8
Use of Trisodium HEDTA in cosmetic formulations

Product category (total formulations in category) (FDA 1998a)	Formulations containing Trisodium HEDTA (FDA 1998a)	Current concentration of use (CTFA 1998a, 1998b, 1999)
Other bath preparations (159)	2	—
Mascara (167)	1	—
Hair conditioners	—	0.1%
Permanent waves (192)	9	—
Shampoos (noncoloring) (860)	13	0.1%–0.7%
Tonics, dressings, and other hair-grooming aids (549)	1	0.3%
Other hair preparations—noncoloring	—	0.1%
Hair dyes and colors (1572)	98	0.1%
Cuticle softeners (19)	2	—
Bath soaps and detergents (385)	17	0.2%–0.5%
Deodorants (underarm) (250)	6	0.1%–0.3%
Other personal cleanliness products (291)	3	—
Skin cleansing preparations (653)	4	—
Face and neck preparations—not shaving	—	0.1%
Moisturizing preparations (769)	2	0.1%
Other skin care preparations (692)	1	—
Total uses/concentration range of Trisodium HEDTA	**159**	**0.1%–0.7%**

GENERAL BIOLOGY

Absorption, Metabolism, Distribution, and Excretion

Wynn, Van't Riet, and Borzelleca (1970) fed rats 1% to 10% Disodium EDTA for 13 weeks (see "Animal Toxicology—Subchronic Toxicity"). During this study, the investigators determined the urinary concentrations of chelated and free calcium, then replicated the assay. These data are presented in Table 9. The remainder of the administered chelate was detected in the feces.

McKay et al. (1994) reported that [^{51}Cr]-EDTA moved passively across the epithelium of the gastrointestinal (GI) tract of dogs. The investigators treated muscle-stripped segments of the stomach, ileum, and colon with 0.5 ml of the chelate at a concentration of 9.0 mM. The rate of flux of the chelate was greatest in the ileum, less in the colon, and least in the stomach. No net accumulation of the probe was observed. In addition, the movement of the chelate across the ileum was not affected by neuronal blockade with tetrodotoxin. The investigators suggested that [^{51}Cr]-EDTA moved from the gut lumen via a shunt pathway.

Foreman and Trujillo (1954) treated healthy adult males (3/group) were treated with ^{14}C Calcium Disodium EDTA to determine its metabolism in humans after topical, IV and IM administration. The investigators 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. A maximum of 5% of the dose was absorbed from the GI tract. After IV and IM injection, 50% of the dose was excreted within 1 hour and 2 1/2 hours, respectively. EDTA mixed with the body's water compartment, but did not pass into erythrocytes and transferred slowly into the spinal fluid compartment. Radioactivity never reached sufficiently large amounts to allow detection in the blood after either oral or topical administration. The maximum activity in the urine after topical administration of ^{14}C Calcium Disodium EDTA was 0.001% of the dose. The only apparent interaction with the body was the combination of EDTA with di- and tetravalent metal ions.

Foreman, Vier, and Magee (1953) used male Sprague-Dawley rats (26) in a series of experiments that determined the distribution and "balance" of Calcium ^{14}C EDTA after intraperitoneal (IP), IV, IM, or oral administration. Female rats were used in a series of experiments in which blood and urine were collected. Urine samples were obtained under ether anesthesia with glass catheters. The animals received 2 cc of saline IP a half hour before the start of each experiment. The dose of Calcium ^{14}C EDTA was 50 mg/kg in 0.2 cc of water (pH 7), which yielded 0.7×10^8 to 1.5×10^8 disintegrations/min of ^{14}C activity. Samples were obtained 1.5, 6, and 24 hours after administration of Calcium ^{14}C EDTA.

The average recovery of the administered dose in the "complete balance studies" (26 rats) was 95.11% ± 5.4%. After IP administration, at 1.5 hours, most of the dose of Calcium ^{14}C EDTA was found in the urine (85.22%), skin (2.38%), blood (1.65%), and kidneys (1.36%); at 6 and 24 hours, most of the dose of Calcium ^{14}C EDTA had been excreted in the urine. Most of the oral dose was recovered in the feces (88.32%) and urine (10.30%) after 24 hours. Administration of Calcium ^{14}C EDTA IV and IM revealed that from 0.5 to 24 hours, the percent Calcium ^{14}C EDTA excreted in the urine increased; at 24 hours, 96.91% and 95.92% of the IV and IM dose, respectively, was recovered in the urine (results are based on the average of 2 to 7 animals). Kidney ligation and IV administration left a considerable amount of activity of Calcium ^{14}C EDTA in the blood even at 26 hours. In assays of expired air less than 0.1% of the ^{14}C activity was exhaled as $^{14}CO_2$. Chromatography confirmed that the compound is not metabolized and passes through the body unchanged (Foreman, Vier, and Magee 1953).

Twenty male Sprague-Dawley rats were divided into four groups of five animals each. Rats in group 1 received IP injections of ^{14}C Disodium EDTA, group 2 received this compound

TABLE 9
Urinary concentrations of calcium and its chelate[a] (Wynn, Van't Riet, and Borzelleca 1970)

	After 8 weeks		After 13 weeks	
Diet	Bound calcium (mg/100 ml)	Free calcium (mg/100 ml)	Bound calcium (mg/100 ml)	Free calcium (mg/100 ml)
Control	—	12.0	—	10.0
		13.0		11.0
1% Disodium EDTA	6.0	12.0	3.3	12.0
	7.0	14.0	4.2	13.6
5% Disodium EDTA	23.0	18.0	11.0	16.5
	30.0	26.0	12.2	18.0
10% Disodium EDTA	18.0	27.0	—[b]	—
	24.0	25.0	9.8	17.8

[a]Data are averages of duplicative determinations for the same two rats per group at different time periods and are representative for each group.
[b]Only survivor in this group.

on depilated skin, rats in group 3 received this compound on depilated and abraded skin (abraded every 2 or 3 cm over treated area), and group 4 was the control group. The specific activity of the ^{14}C Disodium EDTA was 21.6 mCi/mM and it was dissolved in saline to yield a final solution of 50 μCi/ml. Animals that received IP injections got 0.5 ml of this solution, or 25 μCi of ^{14}C Disodium EDTA. Animals that had the compound applied to the skin received 25 μCi of ^{14}C Disodium EDTA in the form of an ointment (modulan, mineral oil, petrolatum, cetyl alcohol 35:21:25:12) spread over an area of 50 cm^2 spread over a sheet of thin polyethylene. This sheet was taped to the trunk of each animal. A collar was fixed around the neck of the rats. All animals were decapitated 24 hours after treatment. The tissue distribution (per 100 mg wet organ weight) of ^{14}C Disodium EDTA 24 hours after IP administration was as follows: liver 577 $\pm$ 13, small intestine 631 $\pm$ 25, large intestine 696 $\pm$ 19, and kidney 1964 $\pm$ 220. Twenty-four hours after application on normal skin the tissue distribution was as follows: liver 6 $\pm$ 4, small intestine 99 $\pm$ 22, large intestine 107 $\pm$ 24, and kidneys 29 $\pm$ 12. Twenty-four hours after application on abraded skin the tissue distribution was as follows: liver 139 $\pm$ 34, small intestine 214 $\pm$ 76, large intestine 309 $\pm$ 115, and kidneys 222 $\pm$ 30 (Furlani and Vertua 1970).

Greiff et al. (1994) instilled a solution containing 5 MBq [^{51}Cr]-EDTA (in 14 ml of isotonic saline) in the nasal cavity of 6 smokers and 12 nonsmokers, and maintained the exposure for 15 minutes. Urine was collected for 24 hours after instillation. The median recovered amount of the chelate in smokers was 0.07 ml, and the median amount in nonsmokers was 0.16 ml. After instillation was repeated with the addition of 0.6% dioctylsodium sulfosuccinate to the solution, the median amount recovered for six nonsmokers increased to 1.13 ml. The investigators concluded that nasal airway absorption was not increased in smokers compared to nonsmokers. The investigators also administered 5 MBq [^{51}Cr]-EDTA and 0.6% dioctylsodium sulfosuccinate in 2.0 ml saline to four separate subjects to determine the GI absorption of EDTA. The mean amount of the chelate recovered in the urine corresponded to 1.4% of the dose.

The glomerular filtration rate was determined by assaying the normal clearance of [^{51}Cr]-EDTA in a study using children (Piepsz, Pintelon, and Ham 1994). The investigators selected 256 patients with past or present urinary tract infections, normal technetium-99M dimercaptosuccinic acid (DMSA) scintigraphy, and normal relative uptake of DMSA (54 to 55 A% for each kidney). [^{51}Cr]-EDTA was injected IV, based on an adult dose of 1.85 MBq. Clearance results were obtained using analysis of one or two blood samples, using 131 and 125 patients, respectively. For the two-sample (second exponential) method, the samples were drawn 2 and 4 hours after injection of the radioactive tracer. Chantler's correction (constant = 0.87) was used to correct the clearance value for having neglected the first exponential. For the one-sample procedure, the sample was taken 115 to 125 minutes after injection. A progressive increase with age of [^{51}Cr]-EDTA clearance was observed, such that the mean adult clearance value was reached between 15 and 24 months. After correction for body surface area, the mean [^{51}Cr]-EDTA clearance increased to 54.6 ml/min/1.73 m^2 by 1 month of age. A plateau was reached at 18 months of age. From 18 to 24 months, the clearance values "were not different from those observed later." From 2 to 17 years, the values were constant; the mean clearance value was 113.9 $\pm$ 24.4 ml/min/1.73 m^2. No significant differences were observed between males and females.

Yang and Chan (1964) reported that rats fed 0.5%, 1.0%, and 5.0% Disodium EDTA for 12 weeks excreted 82.2%, 44.5%, and 45.4%, respectively, of the ingested dose in the urine and feces. The feces contained 99.4%, 98.2%, and 97.5% of the excreted material and the urine contained 0.6%, 1.8%, and 2.5% of the material for the respective doses.

In another study, the equivalents of 1% Disodium EDTA and/or Calcium Disodium EDTA were administered via oral intubation to weanling and adult rats. After 48 hours, the adults given Disodium EDTA excreted 5.3% and 89.9% in the urine and feces, respectively. Adults given Calcium Disodium EDTA excreted 4.6% and 88.5%. Weanling rats given Disodium EDTA excreted 8.5% and 88.6%. Five percent and 3% of the dose were recovered from the intubation syringe and cage, respectively. The investigators concluded that almost all of the administered dose was eliminated from the body within 48 hours of dosing.

The investigators also treated a rat with a single oral dose of 95 mg Disodium EDTA. At regular intervals up to 32 hours after dosing, the GI tract was removed, and the EDTA concentrations in the stomach, small intestine, and colon were determined. The maximum concentrations of EDTA in the small intestine and urine were found approximately 4 hours after dosing. Six percent of the administered dose was excreted in the urine during the 32-hour period. Within 12 hours of dosing, all of the EDTA had passed through the stomach and 93% was recovered in the colon after 32 hours. Similar findings were reported when adult rats were treated with 95 mg/day Disodium EDTA for 7 days. The investigators concluded that EDTA was poorly absorbed and rapidly excreted from the GI tract.

In an additional study, rats were given single doses of 47.5, 95.0, and 142.5 mg Disodium EDTA. For all groups, urinary excretion peaked at 4 hours after ingestion. The amount of EDTA recovered from the urine following a single dose of Disodium EDTA was directly proportional to the dose. The linear relationship suggested that EDTA was absorbed from the GI tract by a process of diffusion (Yang and Chan 1964).

Volf, Vladar, and Seidel (1971) gave IV injections of Calcium Disodium EDTA to female albino Heiligenberg rats. Stock solutions of "unpurified" ^{14}C EDTA was prepared by dissolving this substance in distilled water and adding 1 or 100 μmol of stable calcium. The radiochemical purity of the unpurified solution was 99% and 97% when analyzed on two separate occasions with paper chromatography. Purified EDTA was separated by chromatography and is referred to as "peak 1." The two separate forms of EDTA were labeled at the following positions: *COOH (position 1); *CH_2COOH (position 2); *CH_2CH_2 (position 3).

TABLE 10
Distribution of ^{14}C after IV Injection of Ca- EDTA of two different purities (Volf, Vladar, and Seidel 1971)

Organ	Time after injection (hours)	Treatment			
		1 μM stable calcium		100 μM stable calcium	
		Unpurified EDTA	Peak-1 EDTA	Unpurified EDTA	Peak-1 EDTA
Plasma	1.5	0.079 ± 0.016*	0.055 ± 0.006	0.076 ± 0.030	0.058 ± 0.024
	4	0.022 ± 0.002	0.024 ± 0.006	0.023 ± 0.002	0.028 ± 0.001
	24	0.013 ± 0.002	0.0014 ± 0.0002	0.013 ± 0.001	0.0011 ± 0.0002
Liver	1.5	1.32 ± 0.85	0.24 ± 0.10	0.63 ± 0.06	0.26 ± 0.05
	4	1.03 ± 0.48	0.23 ± 0.09	0.38 ± 0.03	0.21 ± 0.02
	24	0.36 ± 0.09	0.26 ± 0.09	0.21 ± 0.03	0.056 ± 0.008
Skeleton	1.5	0.31 ± 0.07	0.11 ± 0.01	0.43 ± 0.22	0.17 ± 0.10
	4	0.20 ± 0.02	0.045 ± 0.007	0.26 ± 0.03	0.07 ± 0.01
	24	0.11 ± 0.01	0.036 ± 0.009	0.13 ± 0.02	0.031 ± 0.004

*Arithmetic mean of 5 rats ± *SEM* as a percentage of ^{14}C dose.

The amount of radioactive unpurified Calcium Disodium EDTA (100 μM) was greater in the serum, liver, and expired air than the peak 1 fraction. Both unpurified and purified (peak 1) Calcium Disodium EDTA labeled at position 2 were present in the greatest percentages of the dose in serum, liver, and expired air, whereas the edetates labeled at position 1 and 3 came in second and last, respectively. The distribution of the two forms of Calcium Disodium EDTA in plasma, liver, and skeleton are presented in Table 10. The unpurified and peak-1 forms of Calcium Disodium EDTA labeled at position 2 had total serum concentrations of 0.058% and 0.031% of the ^{14}C dose/ml 2 hours after injecting Calcium Disodium EDTA. The peak-1 form labeled at position 3 had a total serum concentration of 0.017% of the ^{14}C dose/ml 2 hours after injecting Calcium Disodium EDTA. The volume of distribution for unpurified and peak-1 Calcium Disodium EDTA is 19.2 ml/g (based on values at 7.5 and 30 minutes after injection) and 21.8 ml/g (based on values at 7.5 and 120 minutes after injection), respectively. The total clearance for unpurified and peak-1 Calcium Disodium EDTA was 1.11 ml/min/g and 0.92 ml/min/g, respectively.

After subcutaneous (SC) injection (1.11 MBq; 0.5 ml), the mean renal clearance of [^{51}Cr]-EDTA was 117.0 ± 20.0 ml/min using 32 normal human subjects. In 20 patients with glomerulopathies, the value was 75.0 ± 38.0 ml/min (Monteiro et al. 1994).

Radioactive Calcium Disodium EDTA was administered IP to rats in doses of 300 to 436 mg/kg/day for 10 days. The total activity recovered in the urine was 66.4% to 92.3%. The fecal excretion was usually less than 5% of the total injected. The mean of the combined activity of radioactive Calcium Disodium EDTA in urine and feces was 94%. The investigators suggest that a rapid and complete elimination of Calcium Disodium EDTA occurs within 24 hours. The activity of both kidneys 24 hours after the last injection was less than 0.1%. Grade 4 microscopic changes were observed in the rats that received the largest dose and grade 2 microscopic changes were observed in the other groups (Doolan et al. 1967).

Intravenous infusion of 4 g of Calcium EDTA or Sodium EDTA slowly over a 4-hour period in four normocalcemic persons resulted in 75% to 88% and 57% to 70% excess excretion of calcium, respectively. Approximately 60% to 80% of the excess calcium was excreted within 4 hours of administering Sodium EDTA. When radioactive doses (dose not specified) of Calcium EDTA were given to three persons, 100% of the chelate was excreted within 24 hours. Calcium EDTA and Sodium EDTA (6 g/day for 6 days) administered orally were poorly absorbed from the GI tract in humans. However, those persons that received Calcium EDTA had increased amounts of calcium present in their stool (Spencer 1960).

Penetration Enhancement

Sodium EDTA (50 and 500 mg/kg; 10 ml/kg IP) enhanced the absorption of two lipid-insoluble acids, heparin and sulfopolyglucan, from the GI tract of rats and dogs (Windsor and Cronheim 1961). Schanker and Johnson (1961) reported that Disodium EDTA (10 mg/ml) also increased the intestinal absorption of neutral, basic, and acidic compounds in the male Sprague-Dawley rat. The chelating agent increased the absorption of [^{14}C]-mannitol and [^{14}C]-inulin from <2% to 7%–11%, the absorption of [^{14}C]-*N*-methyldecamethonium from 2%–3% to 11%–15%, and the absorption of sulfanilic acid from 11%–14% to 26%–32%. Plasma concentrations of the drugs were increased as much as five- or sixfold, compared to controls. Yamamoto et al. (1996) reported that 20 mM EDTA enhanced the intestinal absorption of phenol red (a poorly orally absorbed drug) in male Wistar rats. In the same study, EDTA also caused significant release of protein and phospholipids from the intestinal membrane.

In a study using male New Zealand white rabbits, ^{14}C Disodium EDTA at concentrations of 2×10^{-3} M or 0.5% were applied directly onto the cornea of both eyes. The volume was

25 μl. After dosing with 2×10^{-3} M, significant amounts of EDTA were found in the cornea and conjunctiva; EDTA was also detected in the iris/ciliary body of rabbits killed 20 minutes after treatment. When either glycerol or 0.5% Cromolyn was coadministered with 0.5% EDTA, the concentration of the drug increased in the aqueous humor and cornea and decreased in the iris and conjunctiva. Reduced concentrations of glycerol were observed in the iris and conjunctiva if concentrations of 0.5% to 2% Cromolyn were used as the chelating agent rather than EDTA, but no increase of glycerol was observed in the aqueous humor or cornea. Instillation of calcium after dosing with EDTA reduced concentrations of glycerol in aqueous humor to near that obtained when glycerol alone was instilled, and the glycerol concentration was not decreased in the iris/ciliary body. In this study, 0.5% EDTA promoted the corneal penetration of either glycerol or Cromolyn, although the latter drug (a chelating agent) also increased penetration of glycerol. EDTA appeared to affect the endothelial tissue of the cornea, conjunctiva, and iris/ciliary body, as well as the capillaries of the latter two structures (Grass, Wood, and Robinson 1985).

Disodium EDTA (1 to 10 mM) induced the gradual decrease of membrane resistance in the jejunum of Wistar rats. The primary effect of EDTA was likely at the intercellular junctions. The chelating agent increased the flux rate of sulfanilic acid and decreased the flux rate of L-phenylalanine. EDTA also caused dose-dependent damage to the transport systems of the cells (Yamashita et al. 1987).

EDTA was able to penetrate the cornea via the endothelial side, but penetration into and through the cornea from the epithelial side was considered unlikely due to the charged nature of the EDTA molecule (Grass, Wood, and Robinson 1985).

Using Caco-2 cell monolayers, Tomita, Hayashi, and Awazu (1994) reported that 0.25% Disodium EDTA increased the epithelial permeability of PEG 4000 to 14 times the control value. In calcium-free medium, the EDTA-enhancing effect was increased to 29 times the control value.

Feldman and Gibaldi (1969) reported that 25 mM EDTA increased by ~20% the transfer of salicylate across everted rat intestine, but not the transfer of salicylamide. The effect was greater at pH 7.4 than at pH 6.0, but double the concentration of EDTA did not produce a further increase of the transfer rate at pH 6.0. The investigators concluded that EDTA altered the membrane structure and suggested that the chelating agent would increase permeability of the small intestine to other ionized drugs.

Disodium EDTA at a concentration of 1% (*w/v*; 24 mM) increased the in situ drug absorption of acetazolamide from the small intestine of male Charles River rats when administered with 1% (*w/v*) reduced glutathione (Schoenwald and Ward 1976). Intestinal absorption was increased by 1.5 to 2 times; however, absorption from the stomach was not affected by treatment with EDTA and glutathione. The investigators suggested that Disodium EDTA altered the aqueous permeability of the intestinal epithelium by the chelation of magnesium and calcium ions, thereby separating the epithelial cells.

Disodium EDTA (25 to 50 mM) caused rapid SC absorption of human epidermal growth factor in male Sprague-Dawley rats (Murakami et al. 1993). The chelating agent also increased the plasma area-under-the-curve value of the growth factor, but the inhibitory effect against aminopeptidase activity was small. The investigators suggested that the increased absorption due to EDTA was attributed to the increase of capillary permeability to the growth factor and/or its diffusion through the SC tissues.

Disodium EDTA had no significant promoting effect on the absorption of insulin through the nasal and buccal mucosa of Lewis rats, but it did increase absorption via the rectal route (Aungst and Rogers 1988). Similar results were reported for the absorption of sodium cefoxitin (Nishihata et al. 1985, 1987).

EDTA enhanced the activities of antibacterial agents, particularly against drug resistant gram-negative microbes. The investigators concluded that EDTA increased the permeability of the cellular membranes, thereby increasing the amounts of the antimicrobial agents that penetrated the barriers. EDTA reversed completely the resistance of tetracycline-resistant *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus mirabilis*, and of penicillin- and ampicillin-resistant strains of *P. aeruginosa*. The chelating agent enhanced the activities of polymyxin B sulfate, benzalkonium chloride, and chlorhexidine diacetate against *P. aeruginosa*, and partially reversed resistance of *E. coli* and *P. mirabilis* to chloramphenicol, penicillin, and ampicillin (Brown and Richards 1965; Weiser, Asscher, and Wimpenny 1968; Smith 1970). EDTA was a strong enhancer of Lauricidin against gram-negative bacteria, but had little antimicrobial activity alone (Kabara 1980). Eagon and Carson (1965) reported that EDTA lysed both cell walls and intact cells of *P. aeruginosa* strain 64. Similar results occurred when lysozyme was used in combination with EDTA.

Braide and Aronson (1975) reported that male Long-Evans rats treated with Calcium EDTA (6 mmol/kg/24 h for 48 hours; IV) were more susceptible to the anesthetic effect of pentobarbital. The data suggested that enhanced distribution of the drug had not occurred; rather, Calcium EDTA had an effect on the central neurons.

Mitchell and Gilbert (1984) investigated the effects of pretreatment with EDTA on the cytotoxicity of *E. Coli* CM891 to a variety of mutagens. EDTA (2.5% *w/v*) was added to give a solution of 0.025%. The suspension was shaken and left to stand for 3 minutes at room temperature before each mutagen was added. EDTA pretreatment had no significant effects on the cytoxicity of 2-aminopurine, methylmethane sulphonate, cyclophosphamide, 9-aminoacridine, acriflavine, 2-nitrofluorene, and gentamicin. However, the toxic effects of benzo[a]pyrene and chloramphenicol were increased relative to the controls.

Cytotoxicity

After 24 hours of treatment, a concentration of 5.3 mM Tetrasodium EDTA induced a 50% reduction in cell protein content of cultured Hep G2 cells (Dierickx 1989).

The cytotoxic LD_{50} of Trisodium EDTA in BALB/c-3T3 cells was 1.98 mmol when tested using the coculture survival assay. Trisodium EDTA was classified as moderately cytotoxic (Matthews, Spalding, and Tennant 1993).

At concentrations <100 μM, Tetrasodium EDTA and Cu-EDTA caused cell necrosis and reduced the colony-forming ability of normal rat kidney cells in culture. Cytotoxicity was not observed when the cells were exposed to 100 μM Zn-EDTA or Fe-EDTA, or to 5 μM Tetrasodium EDTA or Zn-EDTA for as long as 7 weeks (Hugenschmidt, Planas-Bohne, and Taylor 1993).

Borenfreund and Shopsis (1985) investigated the cytotoxicity of EDTA in 3T3, Hep G^2, and WI-38 cells. The greatest tolerated doses of EDTA at 24 hours were 620.0 μg/ml and 900.0 μg/ml in 3T3 and Hep G^2, respectively. The concentrations of EDTA that induced 50% inhibition in the uptake of [^{3}H]-uridine after 4 hours were 610.0 μg/ml in WI-38 cells and 200 μg/ml in 3T3 cells. In contrast, the highest tolerated doses of a moderate-severe ocular irritant, sodium dodecyl sulfate (SDS), were 85.0 μg/ml in 3T3 cells and 80.0 μg/ml in Hep G^2 cells. The concentration of SDS that inhibited 50% of uridine uptake was 85.0 μg/ml.

Effects on Mucosa

Yonezawa (1977) induced morphologic changes of the intestinal mucosa in adult male albino rabbits using 10% aqueous EDTA. The rabbits (~3 kg each) were fasted for 24 hours and anaesthetized via IP injection. The intestine was ligated to give a blind loop, into which 20 ml of the EDTA solution was injected. Five minutes later, the intestine was ligated again (3 cm proximal to the blinded section), and further arterial and venous vessels were ligated to cut the blinded section of the intestine. Similar cuts were made and removed at 15, 30, 60, 120, 180, and 240 minutes. The preparations were fixed and stained for microscopic examination. The villi secreted excess mucus from goblet cells from 5 to 60 minutes, but the morphology was not significantly affected. From 120 to 240 minutes, however, the villi had marked epithelial desquamation. Mild lytic degeneration of the lamina muscularis (15 to 120 minutes) and tunica muscularis (120 to 240 minutes), mild to moderate edema of the tela submucosa (15 to 240 minutes), mild cellular infiltration (120 to 240 minutes), and mild (5 to 60 minutes) to severe hemorrhages (120 to 240 minutes) were observed.

Calcium chelating agents such as EDTA were toxic to the urothelium (Oosterlinck et al. 1992) due to the calcium-binding mechanism itself. Dipotassium EDTA buffered with triethanolamine (TEA; pH 8 to 8.5) induced severe lesions to the urothelium of the rat and dog, such that erosion of <20% to >80% of the urothelium was observed (Oosterlinck et al. 1991). At a concentration of 3.125 mM, Dipotassium EDTA caused severe lesions of the urinary bladder mucosa; slow perfusion rates and alternating treatment with physiological solution did not decrease the toxicity (Verplaetse et al. 1985, 1986). Oosterlinck et al. (1992) perfused rat urinary bladder with various chelating solutions for 6 hours at 1.4 ml/h. Magnesium HEDTA (25 to 200 mM, buffered with TEA), Disodium EDTA (12.5 mM, buffered and unbuffered), and Potassium EDTA (12.5 mM, buffered), compared to 0.9% saline, significantly increased the number of lesions. The number of lesions produced by unbuffered Potassium EDTA and buffered or unbuffered Calcium EDTA did not differ from the control. TEA reduced surface tension, and could have enhanced the contact between the urothelium and the calcium ligand, thereby increasing the toxicity.

Braide and Aronson (1974) treated male Long-Evans rats via IV infusion with Calcium EDTA for up to 72 hours at a rate of 6 mmol/kg/24 h. Rats of the control group were infused with 0.9% saline. The permeability of the proximal small intestine was determined at 24, 36, and 48 hours, using [^{51}Cr]-EDTA as a marker. Segments of the duodenum were examined for lesions using light and electron microscopy. Intestinal absorption of the radioactivity did not differ between rats of the control group and those infused with Calcium EDTA for 24 hours. Rats treated for 36 and 48 hours had six- and sevenfold increases in the absorption of the marker, respectively, compared to controls. Within 12 hours of treatment, fulminant lesions in the intestinal epithelium were observed; numerous and large cytoplasmic hyaline bodies and cytoplasmic vacuolization were observed. At 24 hours, advanced "atrophy" was observed such that the connective tissue was "especially prominent." At 36 hours, few intestinal glands were present and the villi were "shrunken beyond recognition." The mitochondria in the crypt and villous epithelial cells were swollen and had intercristal foci of flocculent electron-dense material. Although the outer membrane appeared intact, the endoplasmic reticulum had marked dilatation and the number of free ribosomes was decreased. The investigators concluded that DNA synthesis was inhibited due to interference with cellular respiration, and that the subsequent regeneration of intestinal mucosal cells was prevented.

Miscellaneous Effects

Disodium EDTA inhibited binding of vasoactive intestinal peptide to macrophages obtained from Wistar rats. Inhibition was dose dependent, and the IC_{50} was 5.4 mM. Concentrations of EDTA ≥100 mM "abolished" the interaction between the peptide and the macrophage cell surface (Segura et al. 1996).

At a concentration of 1 mM, EDTA increased the binding affinity of [^{125}I]-angiotensin IV to AT_4 receptors in the bovine adrenal cortex (Jarvis and Gessner 1994). In a study by Peart, Quesada, and Tenyi (1977), 1 to 2 mmol/l EDTA produced a significant increase of renin release in the isolated perfused kidneys of the male Wistar rat. When calcium and magnesium were omitted from the perfusion fluid, EDTA still increased the secretion of renin.

EDTA and HEDTA (500 μmol/kg) alleviated nickel-induced biochemical and trace metal alterations of the serum, liver, and kidneys of female albino rats (Misra et al. 1988).

Gogate et al. (1965) reported that EDTA decreased peripheral utilization of glucose in dogs, possibly via the stimulation of beta cells to produce more insulin, when administered IV every

4 days for total doses up to 5.5 g (500 mg/day in 100 ml 0.9% saline).

Disodium EDTA bound to cell surfaces of human and sheep erythrocyte interfered with agglutination patterns. Binding was enhanced by mild trypsinization of the erythrocytes prior to incubation with the chelating agent. EDTA-bound erythrocytes agglutinated in the presence of divalent cations (Pillai 1982). EDTA also induced platelet agglutination in EDTA-anticoagulated whole blood, possibly via the stabilization of platelet membranes (Ryo et al. 1994).

The membrane permeability and placental transfer of lead in rats was increased with lead-chelate complexes, including complexes of lead with EDTA (McClain and Siekierka 1975). After treatment with EDTA, fetal lead content was approximately 50% greater than with lead alone, but this difference was not significant; in contrast, lead-nitrilotriacetic acid and lead-penicillamine increased placental transfer by 400% to 500% over a 4-hour period. The investigators reported that more rapid maternal excretion limited placental transfer by terminating fetal exposure, thereby decreasing lead-induced teratogenesis.

When eight hypercholesterolemic patients were treated with Disodium Calcium EDTA for 10 days, marked increases were observed in the renal excretion of zinc (10-fold) and lesser increases were observed for cadmium, manganese, lead, and vanadium. The patients were treated with 1.0 to 4.0 g of the chelating agent dissolved in 500 ml of 5% glucose in water. The untreated control group consisted of 24 normal subjects, who each provided a 24-hour sample. Day-to-day variations and the effects of pH changes and dilution were determined using an additional subject. The urinary excretions of titanium, chromium, tin, molybdenum, silver, and nickel were not changed. In addition, the average decrease in plasma cholesterol concentration was 96 mg/100 ml (Perry and Perry 1959).

Calcium Disodium EDTA almost completely inhibited serum dopamine-β-hydroxylase in three lead workers treated IV with 500 mg of the chelating agent. The Cu^{2+}-dependent enzyme converts dopamine to noradrenaline; the mechanism of inhibition involved the chelation of Cu^{2+} ions from the active site of the enzyme. In vitro, the inhibitory concentration (IC_{50}) of Calcium Disodium EDTA was 3.8 mol/l (De Paris and Caroldi 1995). EDTA at a concentration of 200 μl inhibited lipid peroxidation in rat brain homogenates (Kozlov, Ostrachovitch, and Afanas'ev 1994). EDTA inhibited horseradish peroxidase–catalyzed iodide oxidation. This inhibition was both pH and concentration dependent; greater concentrations of EDTA were required to inhibit enzyme activity as the pH was decreased. EDTA was more effective at pH 6 than at pH 4.5. A concentration of 1 mM EDTA completely inhibited iodide oxidation (Bhattacharyya et al. 1994).

The topical application of 0.27 M Disodium EDTA to the cerebral cortex of a rat produced localized epileptic spikes that eventually progressed to general seizures (Harmony et al. 1973).

The intragastric administration of 60 mg/100 g EDTA or another Ca^{2+} chelator, ethylene glycol bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), attenuated both acid-dependent and acid-independent gastric lesions in Sprague-Dawley rats. The chelating agents reduced experimental gastric mucosal damage induced by restraint, cold stress, or treatment with 1.0 ml of 75% ethanol. Gastroprotection was decreased or prevented completely when the rats were vagotomized or adrenalectomized prior to EDTA or EGTA treatment, suggesting that the mechanism of protection involved vagal and glucocorticoid/catecholamine mediation (Glavin and Szabo 1993).

Anghileri (1968) incubated human serum albumin with 0.1 M EDTA (pH = 5.1) and dialyzed against distilled water. It was then incubated with $^{59}FeCl_3$ and the unbound Fe^{3+} ion was eliminated with a cation-exchange resin. The anionic form of the ^{59}Fe (as Fe complexed by EDTA) was adsorbed on an anion-exchange resin. The activity that remained in the liquid is the ^{59}Fe firmly bound to protein. Pretreatment of albumin with EDTA passed through the anion-exchange resin and then incubated with ^{59}Fe, considerably reduced the anionic form of ^{59}Fe, which was still higher than normal albumin. This was used as an indication that EDTA was bound to serum albumin. The percent ^{59}Fe radioactivity bound to albumin, EDTA-pretreated albumin, and EDTA-pretreated albumin passed through the anion-exchange resin was 0.8, 98.7 and 30.6, respectively, and the percent protein was 22.2, 0.5, and 10.6.

In a second experiment human serum albumin was incubated with 0.1 M EDTA (pH = 5.1). After dialysis against distilled water it was incubated with ^{59}Fe (50 and 120 μg $FeCl_3$) in a 0.05 M acetate buffer, dialyzed against an acetate buffer and then saline. The percent radioactivity for albumin pretreated with EDTA was 0.3 and the iron bound to protein was 1.1×10^{-3} μg/mg. The investigators stated that EDTA was bound to the protein and that this bond is broken by anion exchange resin or dialysis.

Effects on Collagen Biosynthesis

Calcium Disodium EDTA increased the urinary excretion of hydroxyproline after IP or IV dosing, indicating that "mature" collagen was degraded (Braide 1984). In this study, male Sprague-Dawley rats were given either IV infusions of 6 mmol/kg/24 h for 48 hours, or IP injections of 4.8 mmol/kg/day for 10 days. Two- to sixfold increases in urine excretion of the imino acid were observed. Rats infused with Calcium Disodium EDTA for 36 hours had gradual decreases of the concentration of hydroxyproline in the urine, after cessation of the infusion. The urinary excretion of trace metals (Zn, Mn, Cu, and Fe) was also enhanced. When Braide (1984) administered the metals to rats and terminated infusion of the chelating agent, hydroxyproline excretion returned to normal. These results indicated that the metals could alter collagen content by making the protein less susceptible to degradation.

Calcium EDTA, when administered IV, caused marked depletion of collagen fibrils in the skin of male Sprague-Dawley rats (Braide, Grill, and Delbello 1988). Rats of the experimental group were infused with 0.9% saline for 24 hours and 6 mmol/kg/24 h Calcium EDTA for the remaining 48 hours. Rats of the

control group were infused with the saline solution for the entire 72-hour period. The rats were anesthetized and the dorsal skin was shaved. A 2-cm^2 skin biopsy was collected from the lumbar region, minced into small pieces, and prepared for examination by transmission electron microscopy. In rats treated with the chelating agent, remnants of collagen fibrils appeared to be in varying degrees of degradation and were loosely arrayed in an extracellular matrix. Most of the fibrils in the matrix had approximately 40% larger average diameters than those of the saline-treated rats. Significant increases (~30%) were observed in the distances between cross striations (D-spacing) of the fibrils. The investigators suggested various explanations for the effects of Calcium EDTA on collagen: (1) lability or reduction of intermolecular crosslinkings, (2) inhibition of crosslinking synthesis or defective synthesis of the macromolecular chains, (3) impairment of DNA, RNA, and protein synthesis due to interference with required zinc, manganese, and iron, (4) direct or indirect degradation of proteoglycans in the connective tissue matrix, and/or (5) inhibition of procollagen synthesis through the chelation of intracellular iron.

EDTA and HEDTA stimulated the enzymatic activity of prolyl hydroxylase by 1.5- to 3-fold at equimolar concentration with ferrous iron in vitro (Takeda et al. 1979). The enzyme hydroxylates proline residues of protocollagen; the reaction requires ferrous iron, α-ketoglutarate, ascorbate, and atmospheric oxygen. Iron chelating agents, therefore, remove the necessary cofactor and inhibit hydroxylation both in vitro and in vivo.

The effect of Disodium EDTA on four strains of *P. aeruginosa* were investigated in vitro in a special protein (0.2% casein, 0.3% beef extract, 0.5% tryptone, 0.1% glucose, and 1.5% agar) and gelatin medium. Disodium EDTA had no inhibitory effect on proteolysis in the protein or gelatin medium at a concentration of 2.5×10^{-3} M. At a concentration of 5×10^{-3} Disodium EDTA inhibited gelatin liquification but not proteolysis. No proteolysis occurred in the media incorporating 10^{-2} M Disodium EDTA. Collagen strips of rabbit achilles tendon were incubated with 10^{-2} M Disodium EDTA. No enzymatic digestion of the strips occurred by the end of 48 hours. However, in collagen strips incubated with no Disodium EDTA hydrolysis was complete from 36 to 48 hours (Wilson 1970).

Female Sprague-Dawley rats were divided into two experimental and two control groups of 10 animals each. A 5-cm dorsal midline skin incision was made in each animal and closed with interrupted 6-0 nylon sutures. Group 1 received 6 mmol/kg/24 h Calcium EDTA IV for 48 hours on postwounding days 13 and 14. Group 2 rats received the same dose of Calcium EDTA IP daily for 14 days. Each control group received saline in a comparable manner to each treatment group. Urine was collected and analyzed in all groups. After 14 days, three 0.5-cm-wide strips were taken from each wound and burst strengths measured. In both treatment groups, urinary hydroxyproline and zinc excretion were enhanced compared to control groups ($p < .001$). A deficiency in serum zinc was observed in both experimental groups. No effect was observed on the burst strengths of the wounds compared to controls (Tobin, Aronson, and Chvapil 1974a).

Tobin, Aronson, and Chvapil (1974b) studied the effects of Calcium EDTA on skin wound healing and urinary hydroxyproline secretion in male and female Sprague-Dawley rats. A 5-cm midline skin incision was made over the cervicothoracic spine and was closed with 6-0 nylon sutures. One group of rats had a colostomy performed in addition to the skin incision. A 2-cm longitudinal incision was made in the ascending colon and closed with 6-0 silk sutures. Twelve days after the skin incision was made, 3 rats/group were infused with either 0.9% NaCl or Calcium EDTA at a rate of 1.08 ml/h and a dose of 6 mmol/kg/24 h. Rats were infused continuously for 48 hours during which time they were fasted.

In a separate experiment, Tobin, Aronson, and Chvapil (1974b) administered 6 mmol/kg/day Calcium EDTA IP to male rats (10/group) for 14 days. Control rats were given 0.9% NaCl. One group received vitamin A and Calcium EDTA and were treated as the controls were except they were given 250,000 IU/kg vitamin A palmitate SC every other day because of the reported synergism between vitamin A and Calcium EDTA on collagen degradation. In another chronic experiment, skin and colon wounds were made and the rats were fed a fluid diet orally to avoid weight loss observed after high doses of Calcium EDTA. This group was dosed IP with 4.8 mmol/kg/day in two divided doses for 14 days. Controls received 0.9% NaCl.

Rats infused for 2 days with Calcium EDTA 12 days after wounding had about four times more hydroxyproline in the urine than controls. Breaking strengths of the skin and gut wounds were not significantly different between control and Calcium EDTA treated rats. More than 50% of the control and experimental animals died when both wounding of the skin and colon were made. The animals treated with 4.8 mmol/kg/day and fed the liquid diet were unclean, depressed, and had diarrhea. However, no change in the breaking strength of either wound was found after treatment with Calcium EDTA. The investigators stated that the unwounded skin of Calcium EDTA treated rats had a significantly lower breaking strength than the wounded skin due to the greater weight loss of this group. However, expression of the breaking strength of the skin wound as a percent of the breaking strength of nonwounded skin demonstrated no different between control and treated rats. The breaking strength of wounded or unwounded skin was not affected by Calcium EDTA treatment alone for 14 days or from vitamin A treatment. However, urinary hydroxyproline excretion was significantly increased. Calcium EDTA administration with or without vitamin A significantly lowered the content of zinc in urine, serum, and hepatic tissue.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

The acute oral LD_{50} of Disodium EDTA was ~3.7 g/kg for both male and female Wistar rats. Signs of toxicity included

ataxia, convulsions, and diarrhea. Changes in tissues were not reported during this study (Hasegawa et al. 1989). Brendel et al. (1953) reported the LD_{50} of Disodium EDTA in mice as 400 mg/kg and the oral LD_{50} in rats of Sodium EDTA was 10.0 g/kg. In studies using dogs and rabbits, the oral LD_{50} values of Sodium EDTA were 12.0 g/kg and 7.0 g/kg, respectively (FDA 1998b). The oral LD_{50} for Trisodium EDTA was 4.0 to 8.0 g/kg in studies using rats (Dow Chemical Company 1987). The oral LD_{50} of Disodium EDTA in rats was >2.0 g/kg. Additional details were not available (BASF 1996a).

The acute oral LD_{50} in Wistar rats of Disodium EDTA was between 2.0 and 2.2 g/kg (Yang and Chan 1964). Rats treated with greater doses of the chelating agent had intestinal hemorrhages, especially in the small intestine. In another study, adult rats were given 95 mg doses of Disodium EDTA at 2-hour intervals via intubation. No signs of toxicity were observed after ingestion of 190 mg EDTA (950 mg/kg), but diarrhea occurred when the total dose was 285 mg (1425 mg/kg).

Intravenous

The effects of Disodium EDTA as a blood preservative was investigated using dogs. Seven dogs per group had blood collected from the femoral artery at a rate of 2 ml/kg/min for a total loss of 30 ml/kg. Thirty minutes later, the blood was added to 4.5% isotonic Disodium EDTA, and the blood was returned to the right femoral vein at rates of 4.6 to 10 ml/kg/min. The final EDTA concentrations were 0.09% and 0.18%. The dogs were evaluated for 15 minutes and 24 hours after the termination of retransfusion. None of the dogs of the low-dose group died as a result of treatment. Venous pressure did not "rise unduly" and arterial pressure returned to normal. Five of the seven dogs of the high-dose group did not have increased arterial pressure, but the venous pressure increased such that the dogs died. In the remaining two dogs of this group, the arterial pressure returned to normal after transfusion and the venous pressure increased only for ~10 minutes, then decreased (Skala 1968; Skala and Pospíšil 1968).

The IV infusion of 4.7% Disodium EDTA (1.65 ml/kg/h) into calves resulted in the chelation of circulating Ca^{2+} such that progressive hypocalcemia occurred (Desmecht et al. 1995).

Payne and Sansom (1964) determined the lethal dose of Disodium EDTA when administered as a single IV injection to rats under ether anesthesia. Disodium EDTA always caused death within 1 minute. The calcium equivalent of the lethal dose was 0.45 mg Ca/100 g rat. Chronic infusion of Disodium EDTA at a rate of 0.05 ml every 20 seconds for 2 to 80 minutes resulted in 0.96 to 1.76 mg Ca/100 g of rat calcium "chelated or removed" before death.

Male, albino, Sprague-Dawley rats were dosed with a single IV injection of 10 and 100 mg/kg radioactive Calcium Disodium EDTA. Animals that were dosed with 1000 mg/kg Calcium Disodium EDTA were given four divided doses over 30 minutes to avoid death and acute toxic effects. The kidneys were removed at 30 minutes, 1, 2, 3, 6, and 24 hours after injection by differential centrifugation experiments, 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. Of the total activity that appeared in the cortex, the fraction that appeared in the sediment increased over the first 3- to 6-hour period at all doses. The percent cortical activity decreased from peak levels by 24 hours. The percent injected activity in the medulla also decreased rapidly during the first 3 hour after administration. Isopycnic density gradient experiments demonstrated no change in density in acid phophatase particles between control and treated animals (Schwartz et al. 1967).

Intravenous administration of Calcium Disodium EDTA in rabbits indicated that the LD_{50} was less than 4 g/kg and greater than 2 g/kg because no deaths occurred at this dose. Additional details were not available (Bauer et al. 1952).

Intraperitoneal

The IP LD_{50} in rats of Tetrasodium EDTA was >2.0 g/kg. Brendel et al. (1953) reported the LD_{50} values of several forms of EDTA after IP injection in mice: Tetrasodium EDTA as 350 mg/kg, Dipotassium EDTA as 450 mg/kg, and Calcium EDTA as 500 mg/kg.

Srivastava et al. (1986) investigated the acute toxicity after IP dosing of EDTA and HEDTA, as well as other polyaminocarboxylic acid chelators, using female albino rats. Ten rats per group were dosed with 266 to 900 mg/kg of either EDTA (0.72 to 2.42 mM/kg) or HEDTA (0.77 to 2.62 mM/kg). All rats of the high-dose groups died as a result of treatment. None of the rats dosed with 266 to 400 mg/kg EDTA or 266 to 423 mg/kg HEDTA died. The LD_{50} values were 512.9 mg/kg EDTA (1.38 mM/kg) and 645.1 mg/kg HEDTA (1.87 mM/kg); the 95% confidence limits were 414.2 to 635.0 mg/kg (1.11 to 1.71 mM/kg) and 528.1 to 788.0 mg/kg (1.53 to 2.29 mM/kg), respectively. All the chelating agents tested in this study caused irritation, convulsions, and aggressiveness. Congestion of the liver, kidneys, and lungs were observed. The investigators concluded that the toxicity of this class of chelators was inversely related to the number of carboxyl groups present in the molecule; for example, triethylenetetraminehexacetic acid with six carboxyl groups was less toxic than EDTA with four groups. The replacement of a carboxyl group with a hydroxyl group decreased the toxicity such that HEDTA was less toxic than EDTA (Srivastava et al. 1986).

The IP LD_{50} of Calcium Disodium EDTA in mice, rats and rabbits exceeded 4.5, 7, and 6 mg/kg, respectively (Bauer et al. 1952).

Schwartz et al. (1966) administered Calcium Disodium EDTA IP in single doses of 1.0 or 2.5 g/kg. Control animals received a corresponding volume of 0.9% saline IP. The kidneys were removed 2 or 24 hours after injection. Microscopic lesions

included vacuolization of predominantly the tubular part of the outer cortex of the kidney, fine vacuolization focally located 2 hours after administration of 1.0 g/kg Calcium Disodium EDTA, and changes in the proximal cells of the affected nephron. Vacuolization became more diffuse throughout the proximal convoluted tubule cells and the vacuoles were much larger 24 hours after administration of 1.0 g/kg Calcium Disodium EDTA. The same lesions were observed with the higher dose of Calcium Disodium EDTA; however, sometimes severe cell damage, characterized by the disruption of the brush border and the presence of free nuclei in the tubular lumens, was noted at 24 hours.

Using differential centrifugation, Schwartz et al. (1966) also examined the renal enzymatic changes (aryl sulfatase, acid phosphatase, and acid ribonuclease) that occurred as a result of a single IP injection of 1.0 or 2.5 g/kg Calcium Disodium EDTA. No changes in the enzyme activity of any fraction or in the total specific activity (total recovered activity/total recovered protein) occurred at either dose at 2 hours. However, significant changes in lysosomal enzymes were observed in both dose groups 24 hours after administration of Calcium Disodium EDTA. Aryl sulfatase activity of the various fractions was significantly decreased at a dose of 1.0 g/kg along with a decrease of total aryl sulfatase activity. Similar results were obtained for aryl sulfatase activity when the dose was 2.5 g/kg. Acid phosphatase activity was decreased in all fractions and the total acid phosphatase activity decreased more than the total aryl sulfatase activity at 1.0 g/kg. In the high-dose group, statistically significant decreases in activity occurred in three fractions of acid phosphatase at 24 hours. Acid ribonuclease activity was evaluated using the *relative* specific activity (the degree of enzyme concentration in the component fraction as compared to that in the combined fraction). Therefore, at the lower dose, a significant decrease occurred in the 300- and 650-g fractions and a significant increase in acid ribonuclease activity occurred in the 150,000-g and supernantant fractions at 24 hours. The high dose of Calcium Disodium EDTA produced a significant decrease in the relative specific activity of acid ribonuclease in the 300- and 650-g fractions and an increase in the 150,000-g and supernatant fractions.

Schwartz et al. (1966) performed separate experiments in which the same solution of Calcium Disodium EDTA that was administered to rats was added to control homogenates, yielding final concentrations of 20 to 200 μmol chelate/ml homogenate. Calcium Disodium EDTA had no effect on the assay of the three enzymes studied.

Short-Term Toxicity

Oral

Kawamata et al. (1980) compared the toxicities of 1% to 5% Disodium EDTA and 5.5% Calcium Disodium EDTA in a 1-month feeding study using 15 rats/sex/group. Some of the rats fed 5% Disodium EDTA died (number not available) and others had marked suppression of body weight gain, decreased leucocyte and lymphocyte counts, decreased calcium serum concentration, and decreased weights of the liver, spleen, and thymus. Microscopic changes included parakeratosis of the esophagus and nonglandular stomach. Similar but milder effects were observed in rats fed Calcium Disodium EDTA.

Fisher 344 rats and B6C3F$_1$ mice (5/species/sex/group) were fed 4640, 6800, 10,000, 14,700, or 21,600 ppm Trisodium EDTA Trihydrate over a period of 7 weeks, followed by a 7-day observation period (see "Carcinogenicity"). Male rats fed $\geq$10,000 ppm and female rats fed $\geq$14,700 ppm had soft stools, considered a sign of compound-related toxicity. After the observation period, the body weights of the treated rats (all doses) were comparable to those of the matched controls. No evidence of organ toxicity was found. Male mice fed 21,600 ppm and female mice fed $\geq$6800 ppm had decreased body weights compared to controls. One male mouse of the high-dose group died. No gross lesions were observed at necropsy (NTP 1977).

When administered in drinking water to white mice, EDTA, Disodium EDTA, Tetrasodium EDTA, and Calcium Disodium EDTA produced changes in the mineral content of the tissues (Sprague 1974–1976). EDTA was administered at a concentration of 3.4 mM (due to its low solubility in water) and the salts were administered at concentrations of 25 mM. The mineral content of bones, kidneys, liver, and muscles was determined using atomic absorption spectrophotometry. Hepatic calcium and renal magnesium concentrations were decreased in mice given Calcium Disodium EDTA. After treatment with Tetrasodium EDTA, calcium was reduced in bone, liver, and muscle; zinc was reduced in the kidneys, muscle, and liver; and magnesium was reduced in bone and liver. Mice given Disodium EDTA had increased renal calcium and magnesium; mice given EDTA had increased muscle calcium; and mice treated with Tetrasodium EDTA had increased renal magnesium. Similar results were reported by Krari and Allain (1991) after oral administration of 1 mmol/kg/day EDTA for 35 days in a study using 42 male Wistar rats.

Intravenous

Male Sprague-Dawley rats ($\sim$19) were infused with a toxic dose of Calcium EDTA continuously over a 48-hour period. Initially, all rats were infused with a solution of 0.9% NaCl for 24 hours. A total dose of 12 mmol/kg Calcium EDTA was administered with plasma concentrations ranging between 300 to 600 μM. The control animals ($\sim$18) received 0.9% NaCl for 72 hours. Rats infused with Calcium EDTA were depressed and had diarrhea and blood in their urine by the end of the treatment. Weight loss was significantly greater in treated animals than in control animals. Weight loss may also have been due to restraint because only minimal amounts of feed were offered at this time. The intestinal tract, especially the duodenum, of rats treated with Calcium EDTA appeared congested. The intestinal wall was thin, the lumen was filled with gas, and the intestinal weight was significantly less than that for control animals (Aronson and Rogerson 1972).

Dogs (20) challenged twice daily with 150, 250, and 500 mg/kg IV had LT_{50} values of 40, 18.5, and 13 days, respectively (Bauer et al. 1952).

The permeability of the small intestine was measured by [^{51}Cr]-EDTA. Absorption of [^{51}Cr]-EDTA was significantly increased in the small intestine of the seven animals evaluated. A marked increase in urinary total hydroxyproline occurred in those animals treated with Calcium EDTA. In the first 12 hours, hydroxyproline content increased almost twofold and by 48 hours, the increase was almost sixfold over the preinfusion level of excretion. Control animals had a slight increase in urinary hydroxyproline related to reduced feed intake and loss of body weight. The excretion pattern of free hydroxyproline differed from total hydroxyproline in that a significant increase was seen at 24 and 48 hours.

In a separate study, three male rats received 50 μCi [^{14}C]-L-proline in three daily IV injections. Four weeks later, two of the rats were infused with Calcium EDTA and the third with NaCl solution. An immediate increase in urinary hydroxyproline ^{14}C radioactivity occurred upon administration of Calcium EDTA. The specific activity of total hydroxyproline excreted in the urine did not change from the 24-hour control period preceding the infusion of Calcium EDTA. A similar pattern was observed in the control rat.

In another study, urine samples from eight rats were selected based on the gross appearance of blood in the urine and were analyzed for amino acid content. Increased excretion of certain amino acids occurred 36 hours following infusion of Calcium EDTA compared to the 12 hours preceding the infusion. Rats that did not have any gross appearance of blood in the urine had either unchanged or slightly increased amounts of amino acids characteristic of collagen present following Calcium EDTA infusion. The increase in proline excretion after Calcium EDTA infusion paralleled the increase in hydroxyproline excretion (Aronson and Rogerson 1972).

Forland et al. (1966) studied the renal excretion of EDTA in 38 dogs. The experiments were performed in the postabsorptive, hydropenic state. Anesthesia was induced and maintained with sodium pentobarbital. Inulin and *p*-aminohippurate (PAH) were given IV throughout the experiment. EDTA plasma concentrations ranged from 0.01 to 0.10 M, given at a rate of 1.6 or 3.2 ml/min in six of the studies. The remaining six dogs received 0.125 M EDTA IV at a rate of 1.6 ml/min. In the six studies, the following solutions were given IV in addition to EDTA: five dogs received Probenecid, 40 mg/kg/h in 0.85% saline, following a 35-mg/kg priming injection; six dogs received *N*′-methylnicotinamide chloride (NMN), 0.25 mg/kg/min in 0.85% saline following a 0.25–0.50-mg/kg priming injection; five dogs received Cyanine 863, 1.5 to 4.0 mg/kg in 0.85% saline over 3 minutes; six dogs received 0.5 M sodium bicarbonate following a 50-ml priming injection; five dogs received 0.03 N hydrochloric acid in 0.85% saline following the injection of 2 g of ammonium chloride in 0.85% saline over 5 minutes. Five additional dogs studied at various plasma EDTA concentrations received 6 g of methionine and 4 g of ammonium chloride daily for 3 days before the experiment and 0.05 N hydrochloric acid was infused at 3.2 ml/min throughout the study. Control animals (27 dogs) were given tubular competitive or inhibitory agents.

The glomerular filtration rate (GFR) was measured using inulin and 60 simultaneous determinations in six dogs; the renal clearance of EDTA closely approximated the inulin clearance. Probenicid selectively inhibited and reversed the organic tubular transport mechanism of PAH. In this study, it failed to alter significantly the renal clearance of EDTA over 30 experimental periods in five dogs. NMN has a functionally separate tubular secretory mechanism that is neither competitive with PAH nor suppressed by Probenecid. Infusion of NMN did not diminish EDTA clearance. Although increases in EDTA clearance were observed in four of six dogs, they were not considered significant. Cyanine 863, a marked and prolonged inhibitor of NMN that does not alter PAH secretion, caused a decrease in the GFR with a comparable decrease in the renal clearance of EDTA. Acidification and alkalinization had no significant effect on renal EDTA clearance. A slightly increased clearance ratio at low plasma EDTA concentrations occurred with pretreatment of methionine and ammonium chloride.

Aronson and Ahrens (1971) administered Calcium Disodium EDTA to female mongrel dogs, female Holstein-Friesian calves (weight 71 to 124 kg) and male Sprague-Dawley rats. The dogs were fasted 18 hours prior to the experiment and hydrated with 20 ml/kg tap water orally 45 minutes before the experiment to produce a mild water diuresis. Sodium bicarbonate (2.5 mmol/kg) or ammonium chloride (dogs, 2.5 mmol/kg and calves, 6 mmol/kg) were given in the hydrating solution when appropriate to dogs and calves. The priming dose was 75 μmol/kg of Calcium Disodium EDTA and 35 mg/kg inulin, followed by the infusion of 1.39 μmol/kg/min Calcium Disodium EDTA and 1.1 mg/kg/min inulin when simultaneous measurements of Calcium Disodium EDTA were made. When clearance of *p*-aminohippurate (C_{PAH}) and tubular maxima (Tm_{PAH}) concentrations were determined priming doses were 7 and 120 mg/kg followed by a constant IV infusion of 0.22 and 2.5 mg/kg/min, respectively. Sustaining solutions were delivered at a rate of 3.5 ml/min. Steady-state conditions were established about 40 minutes after the start of infusion. Probenecid (20 mg/kg) was administered to dogs and calves as a single injection after the third of six consecutive clearance periods in five experiments.

Calves were not fasted and were hydrated with 30 mg/kg tap water orally. The priming dose was the same as used in the dogs, but the infusion rate of Calcium Disodium EDTA was 0.745 μmol/kg/min and 0.5 mg/kg/min for inulin. Determination of C_{PAH} had the same priming injection as the dogs; however, the infusion rate was 0.3 mg/kg/min. Sustaining solutions were delivered at 7 ml/min.

Rats were fasted overnight before the experiment. Incorporation of 20% mannitol in the infusion solution ensured adequate urine flow. A priming dose of 20 mg/kg inulin and 75 μmol/kg Calcium Disodium EDTA was infused with subsequent infusion

rates of 12 mg/kg/min and 3 μmol/kg/min, respectively. The sustaining solution was delivered at a rate of 0.05 ml/min and urine samples were collected at 30-minute intervals 40 minutes after infusion. Urine was quantitatively collected for 6 hours following the renal clearance study. NMN and probenecid were incorporated into the infusion solution and administered to 3 or 11 rats at a rate of 1.2 mg/min, respectively.

The clearance ratio over 65 periods in nine dogs and 45 periods in four calves of simultaneously administered inulin and Calcium Disodium EDTA closely approximated each other over a wide range of pH values but was not significant. The clearance of Calcium Disodium EDTA was independent of the rate of urine flow. The administration of probenecid or NMN did not measurably alter Calcium Disodium EDTA clearance in dogs, calves or rats. The IV administration of 0.3 M sodium acetate at a rate of 7 ml/min to dogs did not enhance Calcium Disodium EDTA clearance. C_{PAH} and inulin clearance (C_{inulin}) measurements in four dogs and three calves were unaffected by Calcium Disodium EDTA. Tm_{PAH} measurements in dogs were also unaffected by Calcium Disodium EDTA. PAH had no significant effect on the volume of distribution or the disappearance halftime of Calcium Disodium EDTA in plasma as studied in four dogs and three calves.

Ahrens and Aronson (1971) studied the effects of Calcium EDTA in female mongrel dogs infused IV with Calcium EDTA at 1.5 mmol/kg/day continuously for 48 hours. Renal function studies used six dogs, three of which had plasma electrolytes measured. Intestinal and renal function were studied in an additional 14 dogs before and after Calcium EDTA administration. The dose for Calcium EDTA in these studies was 3 mmol/kg/day continuously for 36 hours. The dogs had two intestinal cannulas and a urinary bladder cannula surgically placed 2 weeks prior to the experiment. Biopsies of the small intestine were taken at this time. An additional intestinal function study was performed using three dogs 24 hours prior to the start of infusion. Renal function studies measured inulin and PAH clearance, as well as tubular maxima for glucose (Tm_G) and PAH (Tm_{PAH}). Five experiments on the effects of sodium acetate on Tm_{PAH} were also conducted. The priming doses, injected IV, for inulin, PAH, and glucose were 35, 7, and 400 mg/kg, respectively. The sustaining infusion doses for inulin, PAH, and glucose were 1.1, 0.22, and 60 mg/kg/min, respectively. A second primer of 120 mg/kg PAH and sustaining infusion of 1.1 and 2.5 mg/kg/min were used for inulin and PAH.

Signs of toxicity included vomiting, depression, some bloody diarrhea, and moribund state. Metabolic acidosis developed in the last 6 hours of infusion and blood pressure decreased at the end of the infusion. Dogs infused with 3 mmol/kg/day had plasma Calcium EDTA concentrations of 450 to 550 μM. Small, statistically significant reductions, relative to preinfusion of Calcium EDTA, in GFR, renal plasma flow, Tm_G, and Tm_{PAH} were observed. Sodium acetate produced a 12% increase in Tm_{PAH} in the postinfusion period.

Intestinal permeability increased as toxicity developed as evidenced by the appearance of the volume marker [^{51}Cr]-EDTA in the urine after infusion of 3 mmol/kg/day Calcium EDTA. After 36 hours of Calcium EDTA infusion, about 20% [^{51}Cr]-EDTA was absorbed and excreted in the urine while glucose absorption stopped. Net fluxes of water and electrolytes from the duodenal loop were not significantly changed.

Morphologic changes of the intestine of severe congestion and hemorrhage occurred in all dogs given Calcium EDTA. Focal hemorrhages of the colon occurred in one-half of the dogs. Kidneys, adrenal glands, and mesenteric lymph nodes were frequently congested. Biopsies of the intestine taken prior to the experiment were normal. Microscopic changes in tested dogs included degeneration and sloughing of epithelial cells and loss of villous structure, as well as vacuolation and necrosis of the epithelial cells lining the proximal convoluted tubules of the kidneys.

Intraperitoneal

In a 30-day study using female Wistar rats (10/group), the IP LD_{50} was 3850 mg/kg Calcium Disodium EDTA. The rats were treated with 500 to 7000 mg/kg; it was not reported if this was the total dose or the daily dose (Dagirmanjian, Maynard, and Hodge 1956).

Reuber (1969) treated female Buffalo rats with daily IP injections of Calcium Disodium EDTA for 21 days. The test concentration was 20% and the dosage was 500 mg/kg. Each treatment group consisted of 12 rats of varying ages. The control group consisted of six untreated rats. On day 21, the rats were killed and the kidneys, liver, heart, and spleen were removed, weighed, and examined for lesions. Prior to death after 5 to 7 days, 52-week-old rats "lost weight, had brownish and crusty material over the nostrils." Rats, 24-weeks of age when treated, lived for 18 to 21 days. The youngest animals lost weight compared to animals of the control group and died after an average period of 13 days. Survival time for the remaining two groups was not affected, but the mean weights were decreased compared to the control group. As shown in Table 11, mild to severe microscopic changes were observed.

Reuber (1967a) treated male Buffalo rats using the same procedure. Twenty-four- and 52-week-old rats had severe acute tubular necrosis and died within 7 and 5 days, respectively. These rats also had crusty material over the nostrils as did the females of the study reported above. Rats injected at 4 weeks of age had minimal focal hydropic change of the proximal convoluted tubules; rats treated at 12 weeks of age had moderate focal hydropic change within 21 days. Rats treated at 4 and 12 weeks of age survived to the end of the study. The 4-day-old rats treated with Calcium Disodium EDTA that survived to day 13 of the study had extensive, moderate hydropic change, but also had regeneration of the epithelial cells of the proximal convoluted tubules.

TABLE 11
Calcium Disodium EDTA–induced lesions in rats as a function of age (Reuber 1969)

Age	Results (all animals dosed IP with 500 mg/kg EDTA)
4 days	*Kidneys*: Moderate hydropic change of the cells of PCT[a]; tubules lined with regenerating epithelial cells; flattened cells with increased number of nuclei and occasional mitotic figures
4 weeks	*Kidneys*: Small, clear vacuoles in cytoplasm of cells of a few PCT of outer cortex *Liver*: Reversible hydropic change of parenchymal cells
12 weeks	*Kidneys*: Occasional, mild to moderate hydropic change of cells of PCT
24 weeks	*Kidneys*: Moderate hydropic change in focal PCT beneath capsule; smaller tubules deeper in cortex lined by large cells with clear cytoplasm, vesicular nuclei, and nucleoli; no brush border; swollen cells filled lumen *Lungs*: Usually focal congestion and edema of lungs
52 weeks	*Kidneys*: Focally, severe hydropic change of the PCT of the outer cortex; other tubules had mild to moderate hydropic change; small granules of lipid in tubules without hydropic change *Lungs*: Usually focal congestion and edema of lungs

[a]PCT = proximal convoluted tubules.

Similar results were reported by Reuber and Lee (1966) in a study using male Fischer, Buffalo, ACI, and Marshall rats. Marshall rats were the least affected and ACI rats the most affected. Marshall and Buffalo rats had minimal to moderate hydropic swelling of the proximal convoluted tubules. More severe lesions were observed in the tubular cells of both Fischer and ACI rats. However, the ACI rats also had regeneration of the epithelial cells.

Reuber (1967b) carried out an experiment in which 13 4-week-old male rats and 12 4-week-old female rats received daily IP injections of Calcium Disodium EDTA for 21 days. Animals were dosed with a 20% solution of 500 mg/kg Calcium Disodium EDTA. Laparotomy and hepatic biopsy were done on day 22 and biopsies were taken again 21 and 42 days after treatment stopped. Animals were sacrificed on day 63. Hydropic changes of hepatocytes were observed in the first biopsy and this lesion decreased in severity in later biopsies until it was absent at day 63. Foci of inflammatory cells were still present.

Doolan et al. (1967) evaluated the nephrotoxicity of Calcium Disodium EDTA in male and female Long-Evans and Sprague-Dawley rats. Calcium Disodium EDTA (300 to 500 mg/kg) was administered IP daily for 10 consecutive days. Each study had a control group that received the same number of injections of normal saline. Animals were sacrificed 24 hours after the last injection. The radioactive Calcium Disodium EDTA was injected at 300 mg/kg/day for 10 days.

The groups receiving Calcium Disodium EDTA had a 4.3% decrease in body weight (% of initial weight), while the control group gained 6.2% in weight. During the experiment, 6/109 and 1/97 rats died in the Calcium Disodium EDTA group and control group, respectively.

Microscopic changes varied within each kidney and among the animals in a group. Changes were most pronounced in the outer and middle zones of the cortex, and in the mildest form (grade 1) small, clear, randomly dispersed, well-demarcated vacuoles in the cytoplasm of the epithelial cells of the proximal tubules occurred. When grade 2, the changes consisted of increased numbers of vacuoles within a clear cytoplasm. In grade 3, changes in the vacuoles were larger and had replaced the cytoplasm, but did not displace the nucleus. Grade 4 changes included extrusion of cell contents through the disrupted apical border and displacement of the cell nuclei; these changes were not observed very often in groups treated with Calcium Disodium EDTA. The blood vessels and glomeruli appeared normal. The serum urea nitrogen was not significantly affected by treatment with Calcium Disodium EDTA.

Urine volume and protein excretion were measured daily in rats that received Calcium Disodium EDTA, 500 mg/kg, or a comparable volume of normal saline for 10 days. Urine volumes of the treated groups decreased during the first 5 to 7 days, then increased but remained less than that of the control group. The total amount of protein excreted was less than the control group. At microscopic examination, the kidneys had grade 2 and 3 changes.

A marked increase in renal zinc and calcium content and a decrease in manganese was observed in the treated group. The changes in metal content of the kidneys were not correlated with the microscopic changes that had occurred. Additionally, potassium analyses were performed but the treated group values were not significantly different from control values (79.4 ± 3.1 vs. 74.0 ± 2.6). Grade 1 to 4 changes were present in the treated rats, whereas the control rats had no microscopic changes.

Male Sprague-Dawley rats were used in a series of studies to determine the nephrotoxicity of Calcium Disodium EDTA. Initially, two groups of six rats each were given 1000 mg/kg or 3000 mg/kg Calcium Disodium EDTA plus 1% procaine IP for 6 days. Urine was collected and examined and animals were killed on day 6 of treatment. Control animals were injected with procaine alone. Animals that received the high dose developed lesions within 48 hours, whereas those in the lower dose group had signs of renal damage after 4 days of injection. Microscopic lesions in the 1000-mg/kg group included hydropic degeneration

of almost the entire proximal convoluted portion of the renal tubular system with the formation of huge vacuoles and some rupture of the cell walls, and occasional obstructive nephrosis was observed with occlusion of collecting tubules with debris. Rats of the 3000-mg/kg group developed severe hydropic degeneration of the proximal convoluted tubules, no preexisting cells or their component parts were observed, the distal convoluted tubules had parenchymatous degeneration, hydropic change was observed in some cells of the Henle's loops, hyaline and amorphous casts were present in the collecting tubules, dilatation of the proximal tubules was seen, and glomerular tufts appeared edematous with proteinoid precipitate in the glomerular space. Control animals did not develop any lesions (Foreman, Finnegan, and Lushbaugh 1956).

In a subsequent study, Foreman, Finnegan, and Lushbaugh (1956) determined the median effective dose (ED_{50}) in four groups of five rats each that were injected IP daily for 16 days with Calcium Disodium EDTA at doses of 62.5, 125, 250, and 500 mg/kg. Another group of rats was treated with 500 mg/kg/day for 16 days and allowed to recover for 30 days. Finally, a third group of rats was fed 0.25% ammonium chloride and 0.25% sodium bicarbonate and treated with 1000 and 3000 mg/kg Calcium Disodium EDTA to determine if pH of the urine affected the development of renal lesions.

Rats of the 125-, 250-, and 500-mg/kg/day groups developed lesions between 4 and 16 days; however, some animals of this group did not develop any lesions. Rats of the 62.5-mg/kg/day group did not develop any lesions. The ED_{50} was estimated as 203 mg/kg. Animals treated with 500 mg/kg for 16 days and then allowed to recover had normal appearing renal tubules. No differences were found between the urinary findings of treated animals and the controls with respect to pH, volume, and albumin content. However, the urine of the treated animals had casts and epithelial cells.

Microscopic lesions of the lower dose groups were limited to the proximal convoluted tubules. Treatment with 250 mg/kg resulted in microscopic changes in the loops of the tubule adjacent to the glomerulus and further along the proximal convoluted tubule the cytoplasm had many smaller, irregular vacuoles. The 500-mg/kg dose rats had severe changes with half the proximal convoluted tubule having hydropic degeneration. Animals receiving 3000 mg/kg on a normal diet or with ammonium chloride added to the diet died within 3 to 6 days, whereas animals that received sodium bicarbonate died or were moribund by day 6 of the experiment. No additional details were given.

Dalvi, McGrowan, and Ademoyero (1980) determined the effect of Calcium Disodium EDTA on drug metabolizing enzymes in male Sprague-Dawley rats. The animals were divided into seven groups of three rats each, five groups received 0.11 mmol/kg Calcium Disodium EDTA IP daily for 7 days. The remaining two groups were controls and received either the vehicle, normal saline, or DMSO (the vehicle for another chelating agent). The animals were killed 24 hours after the last injection. In an in vitro experiment, these investigators incubated an equimolar (1.0 M) concentration of Calcium Disodium EDTA with liver microsomes from phenobarbital pretreated rats.

Hepatic microsome preparations from the rats pretreated with Calcium Disodium EDTA were incubated with benzphetamine. The rate of metabolism of the drug in the microsomes was significantly lower compared to control animals. However, the amount of cytochrome P450 present was not significantly affected. The amount of cytochrome P450 was not significantly changed when Calcium Disodium EDTA was incubated with microsomes from phenobarbital pretreated rats.

Male albino rats (10 rats/group) were dosed with Disodium EDTA IP over a 4-hour period using an "automatic injection apparatus." Group 1 received 300 mg/kg/day Disodium EDTA, group 2 received the same dose plus 10 mg/day procaine, group 3 received the same dose plus 0.5 mg/day chlorpromazine, and group 4 received 600 mg/kg/day Disodium EDTA. Rats were killed on day 4, 8, 10, 12, or 15. Control rats received daily IP injections of procaine and chlorpromazine equivalent to the dose of the treatment animal for 15 days (Altman, Wakim, and Winkelmann 1962).

Renal tubular damage occurred in all rats treated with 300 mg/kg/day by day 12 and was present in all animals treated for 15 days. At microscopic evaluation of the kidneys, a patchy involvement with severe hydropic degeneration was observed in the epithelium of the proximal convoluted tubules. Some tubular epithelial cells had small, irregular cytoplasmic vacuoles without nuclear changes and other tubules had cells with a single large vacuole and a pyknotic nucleus. Inflammatory changes were absent and the glomeruli and distal tubules were without lesions. Other tissues did not have significant changes in any of the groups. The rats dosed with 600 mg/kg/day developed renal lesions after 4 or 5 days of test material administration. The renal changes were similar to and no more severe than those in the 300-mg/kg/day group. Rats in the high-dose group were moribund after 4 or 5 days and did not survive longer than 6 days. Neither procaine nor chlorpromazine significantly potentiated the renal toxicity of Disodium EDTA in the doses used in this study.

Reuber and Schmieler (1962) studied renal lesions caused by Calcium and Sodium EDTA in 155 male Sprague-Dawley rats. The animals received daily IP injections of either edetate in doses of 250, 400 (only animals that received Sodium EDTA), or 500 mg/kg. The high dose was divided into two IP injections of 250 mg/kg each, given in the morning and afternoon. Surviving animals were killed after 3, 6, 9, 14, and 21 days. Additional groups of rats received 21 daily injections followed by 2 weeks of a nontreatment period and then the rats were killed. Rats dosed with 500 mg/kg Sodium EDTA lost weight, were lethargic, and some died within 1 hour after the injections. The rats that received 400 and 500 mg/kg Sodium EDTA were dead by day 14 and 9, respectively. Rats in the Calcium EDTA groups gained weight throughout the experiment. The bowel was moderately dilated in rats given 400 and 500 mg/kg Sodium EDTA.

Microscopic changes were present in all rats of the 400- and 500-mg/kg groups. The high-dose rats had mild hydropic

changes in one-half of the proximal convoluted tubules in the outer cortex by day 4 and marked degeneration that involved two-thirds of the tubules by days 7 and 8. The 400-mg/kg dose rats had severe changes in one-half of the same area of the kidneys by day 6, and by day 9, two-thirds of the tubules in the entire cortex had marked changes. By 14 days, one-half of the tubules had lesions. The 250 mg/kg dose rats had mild to moderate degeneration in the tubules after 6 days in 30% of the animals, and after 9 days the changes were marked in one-half of the tubules of all rats. Two weeks after withdrawal of the drug, lesions were absent. Tubule regeneration had not occurred at any of the doses of Sodium EDTA.

Lesions developed in 58% of the animals that received 500 mg/kg Calcium EDTA. Mild hydropic changes of the proximal tubules were observed in rats killed during the first 21 days of dosing and occasionally moderate hydropic degeneration was present in the tubules of the outer cortex. After 2 weeks of nontreatment, focal mild subscapular swelling and mild focal regeneration in the loops of Henle were observed. No changes were observed after 21 days or 2 weeks after discontinuing Calcium EDTA.

Subchronic Toxicity

Oral

Eighty male Holtzmann rats (10/group) were fed 1.0% to 10.0% Disodium EDTA for 13 weeks (Wynn, Van't Riet, and Borzelleca 1970). Body weights and average daily feed consumption were determined weekly, and hematologic studies were performed at the end of the 4th and 13th weeks. The urinary concentrations of chelated and free calcium were determined (see "General Biology—Absorption, Metabolism, Distribution, and Excretion"). Half of the survivors were killed and tissue collected for microscopic examination.

Rats fed 5.0% or 10.0% Disodium EDTA had significantly decreased feed consumption (10% to 30% less than controls and 40% to 70% less, respectively) and body weight gains compared to controls. Rats of the high-dose group appeared "emaciated." Rats fed 5% or 10% Disodium EDTA had diarrhea by the third day that lasted throughout the study. Water consumption of rats of the 5% dose group was greater than two times the value of the control group. All of the rats of the high-dose group and 20% of the 5% dose group had priapism within the first 4 weeks to the end of the study. Rats of the high-dose group had slightly decreased hematocrit and hemoglobin values during the 4th week, but these parameters returned to normal by the end of the 13th week. The investigators noted that, although the total concentration of calcium was approximately two times the control value, no evidence was observed of bone erosion or mobilization of calcium reserves. Mortality was 60% in the high-dose group and 20% in the 5% group. The first death of the 10% group occurred during the third week of treatment; one rat of the 5% group was "highly irritable" for several days prior to death and died during a tonic seizure. The LD_{50} was 6.25 mmol/kg. Rats of the high-dose group also had slightly pale livers at necropsy, but no other lesions of toxicity were observed (Wynn, Van't Riet, and Borzelleca 1970).

Groups of three albino rats per sex were fed a low-mineral basal feed (see "Animal Toxicology—Short-Term Toxicity"). 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 (Yang and Chan 1964).

Intravenous

When 10 rabbits were injected IV with 20 mg/kg Calcium Disodium EDTA on alternate days for 6 months, only slight changes occurred in the blood and blood-forming organs (Sroczyński 1966). Erythrocytes with basophilic stippling were observed in the peripheral blood, and the erythroblastic tissue of five of the treated rabbits had lesions of stimulation and impaired maturation. The amount of hemoglobin and the erythrocyte count were not significantly affected, despite slight initial decreases. The reticulocyte count increased slightly as did the amount of serum iron. The amount of copper in whole blood and serum phosphatase activity both were decreased significantly. Other parameters were not affected by treatment with Calcium Disodium EDTA.

Intraperitoneal

Daily IP doses of 0.1, 0.2, 0.3, 0.5, 1.0 g/kg Calcium Disodium EDTA 5 days per week for 14 weeks to Wistar rats (6/dose) produced a graded weight reduction.

When twenty guinea pigs of both sexes were injected IP daily for up to 6 months with 0.8 to 1 ml of 6% Disodium EDTA, the animals died of either a rupture of the ventricular wall or of right ventricular failure (Yamaguchi, Kaku, and Morisada 1993a, 1993b). Those guinea pigs that died of the latter cause had "remarkable dilatation resulting in extraordinary thinning of the wall." Moderate constriction of the pulmonary arteries was observed, with a few narrowly formed elastic elements in the wall. Severe narrowing of the vascular lumens was observed in the peripheral arteries; the constriction was such that individual erythrocytes could "barely" pass through the vessels. Using electron microscopy, the investigators observed irregularly thickened and fragmented internal elastic lamellae of the pulmonary artery. Severe coiling of the internal elastic lamellae and vacuolar formation were observed in the smooth muscle cells. The lamellae were barely stained by tannic acid, a change suggestive of degenerative change in the elastic components. Focal or total expansion and hemorrhage of the vascular wall, as well as plexiform-like lesions of the thin muscular–type arteries, were also observed.

In a subsequent 6-month study by these authors, dilatation of the left ventricle with a moderately thin ventricular wall, as well as right ventricular changes, were observed. The investigators concluded that these changes were due to EDTA-induced

calcium deficiency. The low-calcium content "loosened" the molecular structure of glycosaminoglycans and glycoproteins, which are components of the intercellular matrix and elastic lamellae. This change in the components' viscous characters accelerated the detachment of endothelial cells, but did not adversely affect the joining of endothelial cells (Yamaguchi et al. 1994a, 1994b).

Chronic Toxicity

Oral

When rabbits were treated orally with 250 mg/kg/day Calcium Disodium EDTA, no evidence of toxicity was observed at the end of the 2-year study, or during interim necropsy at 365 and 546 days. Doses of 375 mg/kg/day Trisodium EDTA were not toxic to rats after 721 days of treatment. In a similar feeding study using mice, the only observed effect after treatment with Trisodium EDTA was decreased body weight gain in males. For this study, the lowest-effect level (LEL) was 1125 mg/kg/day, and the greatest no-observable-effect level (NOEL) was 563 mg/kg/day (FDA 1998b).

Fischer 344 rats and B6C3F$_1$ mice (50/sex/group) were fed 3750 and 7500 ppm Trisodium EDTA Trihydrate for 103 weeks (see "Carcinogenicity"). During the study, no compound-related signs of toxicity were observed (NTP 1977).

During a multigeneration (F_3) study, Oser, Oser, and Spencer (1963) fed Calcium EDTA to Wistar-derived rats (25/sex/group). Weanling rats were treated with 0.25% to 4% for 12 weeks, mated, then treated with 0.25% for 18 months. The actual dosages of Calcium EDTA were 50 to 250 mg/kg. Offspring were fed their respective parents' diets. The feeding of the chelate salt had no adverse effects on survival, growth, hematologic parameters, reproduction, or lactation. No gross or microscopic abnormalities were observed.

The same investigators fed 50 to 250 mg/kg Calcium EDTA to groups of four dogs each. All dogs survived to the end of the 1-year treatment period. The feeding of the chelate salt had no adverse effects on growth or organ weights. Evidence was not observed of either osseous changes or other gross and microscopic lesions. Hematologic findings suggested that all dogs treated with Calcium EDTA were in "an even better state of health after 1 year of test feeding than they were initially" (Oser, Oser, and Spencer 1963).

Albino rats (total = 33) were fed diets containing Disodium EDTA (Yang and Chan 1964). Rats of groups 1 and 5 were fed basal diet only. Rats of groups 2, 3, and 4 were fed diets having 0.5%, 1.0%, and 5.0% Disodium EDTA, respectively. Rats of groups 4 and 5 were litter mates born of a dam that had been fed 0.5% EDTA for 8 months. At 12 weeks, the only signs of toxicity were diarrhea and decreased feed consumption in rats of group 4; none of the treated rats died. The study was continued for 2 years. Feed consumption, growth, hematologic parameters, and ash content of the tibia and femur did not differ significantly from controls. Early deaths were attributed to pneumonia; the greatest number of deaths occurred in group 1 and, in decreasing order, groups 2 and 3. None of the rats of group 4 died before scheduled necropsy. No lesions of toxicity were observed at gross and microscopic examination.

Fifty weanling albino rats were given a low-mineral basal feed contained one half the normal salt mixture (Yang and Chan 1964). Rats of groups 1 to 5 were fed basal diet only, 0.5% Calcium Disodium EDTA, 1.0% Calcium Disodium EDTA, 0.5% Disodium EDTA, and 1.0% Disodium EDTA, respectively. At 90 days, the only signs of toxicity were diarrhea and growth retardation in rats of group 5. When the study was continued for a further 115 days, males of group 5 and females of group 3 had reduced growth. An apparent growth stimulation was observed in males of group 4 over the first few months. Feed consumption did not differ significantly between groups, although the most favorable feed efficiency ratio occurred in rats of group 3. The only signs of toxicity during the study were diarrhea and "anemic appearance" for rats of group 5. These rats had statistically decreased erythrocyte and leucocyte counts, but the individual values were within the normal range for an adult rat. Rats of group 5 also had longer blood coagulation time, compared to controls, which indicated that complexing of the free ionic calcium in the blood could have interfered with the coagulation mechanism. In addition, the group 5 serum calcium values were slightly increased compared to controls. The total ash content of tibia was normal for groups 1 to 4. In group 5, the ash content of the tibia was decreased, indicating possible decalcification, and "considerable erosion" was observed in the molars. Males were more susceptible than females to dental erosion and the number of rats with erosion could be correlated with the decreased ash content of the tibia. In contrast, no evidence of erosion was observed in rats fed normal basal diet (containing adequate minerals) to which 1% Disodium or Calcium Disodium EDTA had been added. No gross lesions were observed at necropsy and no lesions were found at microscopic examination of the liver, kidneys, and spleen. Rats of group 5 had "somewhat dilated" hepatic sinusoids and a slightly increased number of Kupffer cells; the renal tubular epithelium had "an indistinct appearance" in some of the treated rats.

Intraperitoneal

The time-to-50% mortality (LT_{50}) of Calcium Disodium EDTA was 78 days for rabbits and cats when given daily (5/week) IP injections of Calcium Disodium EDTA at doses of 0.5 g/kg (rabbits) and 0.25 g/kg (cats).

Skin Irritation and Sensitization

Disodium EDTA was classified as a nonirritant in a primary skin irritation test in rabbits. Additional details were not available (BASF 1996a).

Five male guinea pigs were injected intracutaneously with a volume of 0.1 ml, then 9 volumes of 0.2 ml Disodium EDTA on alternate days. A challenge injection (0.1 ml) was given 2 weeks after the last induction injection, and the treatment sites were

evaluated 24 hours later. The guinea pigs had no signs of allergic reactions (Yang and Chan 1964).

Henck, Lockwood, and Olson (1980) determined the skin sensitization potentials of Trisodium EDTA and ethylenediamine in a study using male Hartley guinea pigs (10/group). The positive control was the diglycidyl ether of 2,2-di(*p*,*p*′-hydroxyphenyl)propane. The three test compounds were applied as 10% solutions in dipropylene glycol methyl ether and Polysorbate 80 (9:1). Test volumes of 0.1 ml were applied topically to the clipped and depilated backs of the guinea pigs, four times in 10 days. At the time of the third application, 0.2 ml Freund's adjuvant was injected intradermally adjacent to the application site. The guinea pigs were challenged on clipped flanks 2 weeks after the fourth treatment. Animals that had received Trisodium EDTA or ethylenediamine during the induction phase of the study were challenged with both compounds, one per flank. The treatment sites were examined for irritation and sensitization at 24 and 48 hours. Under the conditions of this study, EDTA did not cause sensitization in any of the 10 guinea pigs and did not cross-sensitize with ethylenediamine. Ethylenediamine caused slight to marked erythema and slight edema, as well as sensitization in all 10 guinea pigs.

Ocular Irritation

Disodium EDTA was classified as a nonirritant in a primary mucous membrane irritation test using rabbit eyes. Additional details were not available (BASF 1996a).

Meyer and McCulley (1991) investigated the effects of chelation on biocompatibility of various pharmaceutical vehicles using rabbit stratified corneal epithelial cultures. EDTA (0.05% and 0.1%) was added to balanced salt solutions, acetate/citrate-buffered saline, phosphate-buffered saline, and saline at both pH 5 and 7.5. Acute cytotoxicity was indicated by the percent release of [^{3}H]-nucleotides after a 32-minute exposure and surface damage was evaluated by phase contrast microscopy. Protracted cytotoxicity was indicated by the residual release of ^{3}H, evidence of penetrating damage (perforation or necrosis of deeper layers of stratified tissue), tissue loss, and decreased protein synthesis. The exposure time causing 50% tissue damage (CDT_{50}) was determined for both acute and protracted cytotoxicity.

Balanced salt solutions (both pHs) were not cytotoxic, with or without the addition of EDTA. At pH 5, EDTA slightly decreased the toxicity of the acetate/citrate-buffered vehicle, but enhanced the toxicity of the vehicle at pH 7.5. EDTA did not increase the toxicity of the phosphate-buffered solution or the unbuffered saline solution at pH 5, but the cytotoxicity was increased at pH 7.5. EDTA slightly reduced the toxicity of the acidic acetate/citrate-buffered solution, but at alkaline pH, the chelating agent increased both acute and protracted injury. In general, the acidic solutions were relatively nontoxic compared to the acidic acetate/citrate-buffered solution, and EDTA was tolerated more in acidic solutions than in alkaline solutions. The investigators suggested that EDTA either inhibited the penetration of citrate into the epithelial cells or competitively inhibited the binding of divalent ions to citrate (also a chelating agent), thereby decreasing citrate's effect on intracellular pH.

Grant (1952) dropped a 1 M solution of EDTA onto the rabbit cornea; the solution produced pain and chemosis. Repeated exposure for 15 minutes of de-epithelialized eyes caused marked swelling of the corneal stroma, intense conjunctival chemosis with small conjunctival hemorrhages, and hemorrhagic iritis. No damage occurred when a 0.1 M solution was dropped once on the normal human cornea. A 0.1-M solution dropped continuously for 15 minutes on rabbit cornea with the corneal epithelium removed caused a little more corneal edema, chemosis, and hyperemia of the iris than did the removal of the epithelium itself. Healing was complete in 3 days, which was comparable to de-epithelialized controls. The exposed corneas were as clear as the control corneas. A 0.01-M solution of EDTA dropped on the human cornea produced almost no discomfort. Repeated droppings for up to 15 minutes caused no damage, only mild, pale edema of the conjunctiva. The same concentration dropped on de-epithelialized rabbit corneas caused mild, pale conjunctival edema and mild edema of the corneal stroma. The eyes returned to normal in 2 to 4 days. A 0.01-M solution injected intracorneally produced a pronounced, bluish swelling of the cornea at the injection site that disappeared in 2 weeks. A 0.01-M solution of EDTA injected into the anterior chamber caused a diffuse corneal edema and marked hyperemia of the iris and conjunctiva. These observations led the investigators to conclude that a 0.01-M neutral solution of EDTA was safe and nonirritating for dropping on the denuded human cornea for 15 minutes, but not for injection into the globe.

Sugar and Waltman (1973) injected intrastromally varying concentrations of a 0.1 ml solution of Calcium Disodium EDTA into the central cornea of 10 rabbit globes. The animals were sedated with chloropromazine and anesthetized with topical proparacaine. The eyes were examined daily and scored on a scale of 0 to 4+. A 0.2-M solution of Calcium Disodium EDTA caused very mild persistent central haze and edema. This same solution of Calcium Disodium EDTA produced a 0.2 score for opacity and edema on day 2 and 0.3 and 0.2 scores for opacity and edema, respectively, on day 10.

In a second experiment, these investigators denuded the corneas of rabbits by repeated scraping. One drop of a 20% 0.2-M solution of Calcium Disodium EDTA was applied to each of 10 corneas four times daily for 10 days. A "balanced salt solution" was applied the same way to 6 corneas. Topical EDTA plus polymyxin-B-bacitracin or EDTA alone was applied to an additional 14 corneas (7 corneas each). Topical applications of 0.2 M Calcium Disodium EDTA caused a diffuse superficial groundglass appearance in the stroma of 8/10 corneas at 6 and 10 days. The haze persisted in 5/8 eyes 2 weeks after stopping the treatment. None of the animals that received the balanced salt solution had corneal opacity. No significant differences occurred after 5 days between the groups treated with the EDTA plus antibiotic or EDTA alone.

TABLE 12
Malformations in rats treated with Disodium EDTA (Swenerton and Hurley 1971)

Group	No. rats mated	Treatment	Exposure on GD[a]	% gross malformations
1	11 (11/11)[b]	0% Na_2EDTA, 100 ppm Zn	Control	0
2	5 (5/5)	2% Na_2EDTA, 100 ppm Zn	0–21	7
3	8 (0/0)	3% Na_2EDTA, 100 ppm Zn	0–21	*NA*
4	11 (10/8)	3% Na_2EDTA, 100 ppm Zn	6–14	87
5	16 (16/11)	3% Na_2EDTA, 100 ppm Zn	6–21	100[c]
6	8 (7/7)	3% Na_2EDTA, 1000 ppm Zn	6–21	0

[a]GD, gestational days.
[b]No. in parentheses = no. rats with implantation sites/no. rats with living young at term.
[c]% of each gross malformation is indicated in the text.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Pregnant Sprague-Dawley rats were fed 2% to 3% Disodium EDTA, with and without zinc supplementation (Swenerton and Hurley 1971). Feed and water were available ad libitum.

Results for the different treatment groups are presented in Table 12. All dams fed EDTA had moderate to severe diarrhea. The fetuses were removed by cesarean section and fixed for gross examination on gestational day (GD) 21. The eyes and brain were examined in razor cut sections of the head, but the fetuses were not examined for other internal abnormalities. All dams of group 2 had living young at term, and the litter size did not differ from the control. The incidence of malformations was 7% for offspring of this group. None of the mated females of group 3 had grossly visible implantation sites at term. Most of the mated females of groups 4 and 5 had implantation sites, but nearly one-half had dead or resorbed fetuses. The incidence of malformations in the fetuses of group 4 was 87%. The litter size for group 5 was reduced by >50%, and the mean body weight was decreased significantly (1.8 g compared to 5.3 g for the control offspring). Dams of group 5 had 83 living young at GD 21. These offspring all had gross malformations: cleft lip (incidence = 1.2%), cleft palate (57%), brain malformations (hydrocephalus, anencephalus, hydranencephalus, or exencephalus; 44%), micro- or anophthalmia (18%), micro- or agnathia (63%), clubbed legs (92%), fused or missing digits (57%), or tail malformations (curly, short, or missing tail; 98%). In contrast, none of the 81 offspring of group 6 had gross malformations. The investigators concluded that Disodium EDTA was teratogenic to rats, possibly due to an induced deficiency of zinc (Swenerton and Hurley 1971).

Brownie et al. (1986) evaluated the teratogenic potential of Calcium EDTA at dosages one-half to twice the recommended maximal daily dose for children (1500 mg/m^2/day). ZnCaEDTA and ZnEDTA were dosed at 8 and 20 mmol/m^2/day, respectively. The investigators injected SC pregnant Long Evans rats (20 for each group) with Calcium EDTA, ZnEDTA, or ZnCaEDTA at 12-hour intervals on GDs 11 to 15. Twelve dams per group were used for plasma and hepatic zinc analysis and for evaluation of intestinal and renal lesions; six per group were killed on GD 16, and the remainder were killed on GD 21. For the other dams, the pups were removed by cesarean section on GD 21 and were examined for skeletal and visceral abnormalities.

Treatments received by the different groups are described in Table 13. Females of group 2 had decreased weight gain and dams of groups 3 and 4 had diarrhea as well as decreased feed consumption, water intake, urine production, and weight gain. One rat each of groups 3 and 4 died on GD 17, also animals of those two groups had mild renal lesions. No other microscopic abnormalities were observed. All fetuses were resorbed in one and four dams of groups 3 and 4, respectively. Thirteen of 19 litters of group 4 had at least one resorbed fetus, compared to 9 of 30 litters of the control group (group 11). Fewer live births per female were reported in group 4, compared to controls. Offspring of females treated with 4 to 8 mmol/m^2/day Calcium EDTA had dose-related decreases of mean body weight and mean crown-rump length. The developmental abnormalities observed in this study are described in Table 13. In this study, maternal and fetal toxicity were not observed for groups treated with ZnEDTA or the low dose of ZnCaEDTA. Submucous cleft was observed in 6 of 20 litters of group 5. The investigators concluded that Calcium EDTA was teratogenic to rats at nonmaternotoxic doses (except for decreased weight gain) and that zinc had a protective effect when incorporated into the chelate (Brownie et al. 1986).

Kimmel (1975) reported gonad dysgenesis in offspring of pregnant CD rats treated with EDTA on GDs 7 to 14. EDTA was administered to the rats in feed, by gavage or by SC injection at concentrations of 1000 mg/kg/day, 1250 mg/kg/day, or 325 mg/kg/day, respectively. The feeding of EDTA caused maternal toxicity, but none of the females died. Seventy-one percent of the offspring had severe malformations similar to those observed by Swenerton and Hurley (1971). Of the dams treated by gavage, 36% died as a result of treatment; malformations were observed in 21% of the offspring. When injected SC, EDTA killed 24%

TABLE 13
Teratogenic effects of Calcium and Zinc/Calcium EDTA (Brownie et al. 1986)

Group (no. litters)	Treatment	Abnormalities: no. observed (no. litters)
Control		Submucous cleft: (palatine bone space) 1(1); missing sternebrae: 7(4); split sternebrae: 7(4); no other abnormalities were observed
1 (20)	2 mmol/m^2/day CaEDTA	Cleft palate: 1(1); submucous cleft: 7(4); curly tail: 1(1); missing sternebrae: 8(2); split sternebrae: 7(4)
2 (20)	4 mmol/m^2/day CaEDTA	Cleft palate: 10(2); submucous cleft: 24(6)[a]; curly tail: 7(1); adactyly-syndactyly: 7(1); missing sternebrae: 14(5); split sternebrae: 13(5)
3 (18)	6 mmol/m^2/day CaEDTA	Cleft palate: 25(3)[a]; submucous cleft: 20(5)[a]; curly tail: 18(2); adactyly-syndactyly: 18(2); missing sternebrae: 14(5); split sternebrae: 12(6); fused sternebrae: 2(1); brachygnathia: 1(1)
4 (15)	8 mmol/m^2/day CaEDTA	Cleft palate: 47(8)[a]; submucous cleft: 7(4); curly tail: 31(7)[a]; anasarca: 22(5)[a]; adactyly-syndactyly: 30(8)[a]; hydrocephalus: 2(2); hernia: 2(2); wavy ribs: 16(2); missing sternebrae: 25(8)[a]; split sternebrae: 3(2); fused sternebrae: 1(1); brachygnathia: 6(3)[a]; missing centrum vertebrae: 10(2); split vertebrae: 3(1); fused vertebrae: 1(1)
5 (20)	8 mmol/m^2/day ZnCaEDTA	Curly tail: 2(1); missing sternebrae: 1(1); split sternebrae: 10(6); no other abnormalities were observed
6 (20 dams treated)	20 mmol/m^2/day ZnCaEDTA	Submucous cleft (palatine bone space): 16(6); missing sternebrae: 2(2); split sternebrae: 15(8); no other abnormalities were observed
7 (20)	8 mmol/m^2/day ZnEDTA	Missing sternebrae: 2(2); split sternebrae: 11(7); split vertebrae: 1(1); no other abnormalities were observed
8 (20 dams treated)	20 mmol/m^2/day ZnEDTA	Submucous cleft: (palatine bone space): 4(1); split sternebrae: 10(4); no other abnormalities were observed
9 (20)	Saline, pair-fed to group 4	Submucous cleft (palatine bone space): 6(3); anasarca 1(1); missing sternebrae: 2(2); split sternebrae: 5(4); no other abnormalities were observed
10 (20 dams treated)	Hypertonic saline	Missing sternebrae: 3(3); split sternebrae: 7(7); no other abnormalities were observed
11 (30)	0.9% NaCl solution	Submucous cleft: 1(1); missing sternebrae: 7(4); split sternebrae: 7(4)

[a]Significantly different from control (χ^2 test of homogeneity with continuity correction, at the 5% level, for the 2 × 2 contingency table containing no. litters with and without abnormality for the control and treated groups).

of the dams, but was not teratogenic. Typically, the sex of the malformed offspring could not be determined except by microscopic examination. The gonads were not well differentiated and the number of germ cells was decreased significantly. Male offspring had small, cryptorchid testicles with few seminiferous tubules; few spermatogonia were observed. The investigator reported that the testicular changes were similar to those reported in the offspring of zinc-deficient rats and suggested that the observed changes were the result of the effect of EDTA on the concentration of the metal.

In another study, zinc concentrations were not changed significantly in maternal plasma and embryos during 3% EDTA treatment on GDs 7 to 14 (Kimmel and Sloan 1975). Within 2 days after termination of treatment, however, the zinc concentrations were increased 20% to 40% compared to controls. In studies where ^{65}Zn was injected (IV) on GD 6, little or no change occurred after GD 10, whereas the zinc concentration decreased significantly in control rats. The investigators suggested that EDTA bound free zinc, making it unavailable for exchange with incorporated zinc. EDTA produced a zinc deficiency that resulted in teratogenic effects.

Apgar (1977) 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 GD 18 underwent stress at parturition, evidenced as prolonged parturition and/or failure to consume the afterbirth. Stress, hematocrit 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.

Schardein et al. (1981) evaluated the teratogenicity of EDTA and its disodium, trisodium, calcium disodium, and tetrasodium salts using CD albino rats. Twenty inseminated rats were intubated with 2.3 to 2.4 ml of the test compounds (equivalent to 1000 mg/kg EDTA), equally divided, twice daily on GDs 7 to 14. The rats of the vehicle control group received 1.0 ml/kg doses

of phosphate buffer twice daily. An additional untreated group served as the control group.

Females of all treatment groups had diarrhea; the incidences were 80% (EDTA), 65% (Disodium EDTA), 35% (Trisodium EDTA), 10% (Calcium Disodium EDTA), and 90% (Tetrasodium EDTA). On the first day of dosing, three females of the EDTA group and one of the Trisodium EDTA group had transient episodes of decreased activity. Three females given Disodium EDTA died during treatment, but had no gross abnormalities. The females were killed on GD 21, and each fetus was examined for external anomalies. The test compounds did not significantly affect litter size, postimplantation loss, or the sex ratio compared to controls. One-third of the fetuses were inspected for visceral abnormalities and two-thirds were examined for skeletal abnormalities. No pattern was observed regarding treatment with a particular compound or the incidence of anomalies. The investigators concluded that EDTA and its salts were not teratogenic under the conditions of this study, even at maternally toxic doses (Schardein et al. 1981).

Yang and Chan (1964) reported that four groups of weanling rats (two males and four females per group) were given 0.5% to 5.0% Disodium EDTA in a feeding study. The rats were mated after 100 days, and mating was repeated 10 days after weaning the first litters. Rats of the high-dose group failed to produce litters, even though they had been mated for 2 months. Rats of the untreated control group and those fed 0.5% to 1.0% of the test chemical had normal first and second litters.

During an in vivo–in vitro teratogenicity study, pregnant Alderley Park strain I rats were injected (IP) with various chemicals, including 40 mg/kg Sodium EDTA (Flint, Orton, and Ferguson 1984) on GD 12. The uteri were removed 16 hours later, and the embryonic limb bud and midbrain cells were cultured. Growth was determined by assaying the total protein per cell island, and differentiation was determined as the number of differentiated foci and the incorporation of ^{3}H-γ-aminobutyric acid (GABA) or $^{35}SO_4$. The vehicle control was 0.5% aqueous polysorbate 80 for rats treated with Sodium EDTA. Sodium EDTA increased the number of differentiated foci in both types of cultures; the values were 105% and 128% of the control values for the midbrain and limb bud cells, respectively. Growth was decreased in the midbrain cells (67% of control) and increased in the limb bud cells (132%). The incorporation of [^{3}H]-GABA and $^{35}SO_4$ were 79% to 80% of the control values for both cultures. Sodium EDTA inhibited culture development by less than 20%, but was classified as a teratogen under the conditions of this study.

Disodium EDTA had no effect on either the testicular or epididymal weights and microscopic structure when the salt was administered in water to Swiss albino (CFT) mice at doses of 5 to 15 mg/kg/day for 5 consecutive days. Caudal sperm counts and the incidence of sperm-head abnormalities did not differ from controls. In addition, the incidence of postimplantation embryonic deaths was not increased after males treated with 10 mg/kg/day for 5 days were mated with untreated females (Muralidhara and Narasimhamurthy 1991).

In an oral study using CD-1 mice (Gray and Kavlock 1984), resorptions and malformations were not observed in offspring of dams treated with 1000 mg/kg/day EDTA on GDs 8 to 12. The chelating agent also had no effect on litter size, sex ratio, organ and body weights, or the percentage of viable offspring, compared to controls.

Oser, Oser, and Spencer (1963) reported that feeding a diet containing 0.25% to 4% Calcium EDTA had no adverse effects on reproduction or lactation during a multigeneration study (F_3) using Wistar-derived rats (see "Animal Toxicology—Chronic Toxicity").

In an oral study using Alpk:AP (Wistar-derived) rats, treatment with 1 g/kg EDTA on GDs 7 to 16 did not cause differences in the group mean weight changes of the dams, number of viable litters, mean litter size, number of live pups, percentage survival of the pups, mean pup weights, and mean percent weight gain per litter, compared to treatment with physiologic saline (Wickramaratne 1987).

Gasset and Akaboshi (1977) investigated the embryopathic effects of EDTA at the concentrations used in ophthalmic drops. Pregnant albino rabbits (four per group) were treated with 0.1% and 3% EDTA, freshly prepared from a 5% solution of Disodium EDTA. Two drops of the test concentrations were instilled into the conjunctival sac six times per day from GDs 6 to 18. The rabbits were killed on day 29, and all fetuses were removed and examined for external, skeletal, and microscopic abnormalities. The rabbits of the low-dose group had 23 live fetuses and 3 dead, aborted, or resorbed fetuses were also observed. The rabbits of the high-dose group had 8 live fetuses and 19 dead, aborted, or resorbed fetuses were also observed. Neither group had living, anomalous fetuses, and the percentages of normal progeny were 89% and 30% for the low- and high-dose groups, respectively. If the levels of test compound given were fully absorbed by the ocular tissue, the doses were 0.3 to 9 mg/day. The investigators concluded that 0.1% to 3% EDTA was nonteratogenic, but the larger dose had a significant embryocidal effect.

The effect of the route of administration on teratogenicity was evaluated by Kimmel (1977) using pregnant CD rats. On GDs 7 to 14, EDTA was administered in the diet at doses of 954 mg/kg/day (3% of diet; 42 dams), by gastric intubation at 625 or 750 mg/kg/day (twice daily; 22 and 8 dams), or subcutaneously at doses of 375 mg/kg (25 dams). Thirty-eight, 20, and 14 females served as untreated controls for the three routes of administration, respectively. The females were killed on GD 21, and only live fetuses were examined for external, visceral, and skeletal malformations. When rats were fed EDTA, none of the dams died, but maternal toxicity was severe. Marked maternal weight loss, decreased feed consumption, and severe diarrhea were reported. Seventy-one percent of the offspring had malformations, and the average fetal weight was decreased significantly. Of the rats given EDTA IP, 87.5% died after dosing with 750 mg/kg/day, and 36% died after dosing with 625 mg/kg/day. The number of

resorptions was not increased significantly compared to controls. Fewer young (20.5%) of this group were malformed, compared to the offspring of dams fed EDTA. When administered SC, the females were in shock and had vocalizations that indicated pain, 24% died. Maternal and fetal body weights were decreased significantly. The incidence of malformations was not significantly different from controls. For all groups, the typical gross malformations included cleft palate, umbilical hernia, phocomelia, micrognathia, clubfoot, short curly tail, meningocoele, and microphthalmia. Visceral abnormalities included great vessel abnormalities, interventricular septal defects, small or missing lung lobes, missing thymus, small kidneys with hydronephrosis and hydroureter, and small, undifferentiated gonads situated lateral to the kidneys. Skeletons of deformed fetuses had extreme dysplasia, including shortened, missing, or wavy ribs; misaligned and fused centra; and anomalies associated with external defects.

Fetal resorptions were not reported after pregnant Sprague-Dawley rats were fed 3% Disodium EDTA from GDs 3 to 13. In contrast, another copper-chelating agent, disulfiram, induced fetal resorptions when fed to the rats at a concentration of 1% (Salgo and Oster 1974).

Koshakji and Schulert (1973) studied the effects of Disodium EDTA on pregnant Sprague-Dawley rats. Day 0 of pregnancy was designated as the day spermatozoa were detected in the female's vagina and the presence of a copulatory plug further confirmed pregnancy. On day 9 of pregnancy, 380 mg/kg of Disodium EDTA was administered subcutaneously to 10 rats. Marked maternal body weight loss during the advanced stages of pregnancy, as well as loss of appetite, complete relaxation, weakness, drowsiness, muscular limpness, inactivity, and accelerated respiration rate occurred. Rats also had signs of shock, pain, and abnormal behavior during and immediately after injection. Although no maternal loss or fetal malformations occurred after treatment, administration of Disodium EDTA produced much fetal resorption. Of 115 implantations, only 30 fetuses were born alive. The mean fetus weight was not significantly different from controls. Significantly increased amounts of Zn, Fe, and Mn were observed in the urine when Disodium EDTA was administered on day 9 of pregnancy. Significant decreases in the maternal-fetal uptake of Zn, Fe, and Mn occurred 6 hours after 200 mg/kg Disodium EDTA was administered to four 16-day pregnant rats. Significant decreases were observed in whole blood, kidneys, liver, fetus, yolk sac, fetal-placental unit, and urine at this time. The investigators suggested that the teratogenic activity attributed to Disodium EDTA could be a result from metal chelation, subsequently resulting in metal suppression that deprived the developing embryo of these minerals.

EDTA had a synergistic effect with gramicidin (an antiviral and antibiotic) in inhibiting sperm motility and cervical mucus penetration in vitro (Bourinbaiar and Lee 1996). At a concentration of 0.1%, EDTA decreased the IC_{100} of gramicidin from 4 μg/ml to 10 ng/ml; the gramicidin/EDTA combination was 100,000 times more potent than a nonoxynol-9/EDTA combination in a sperm penetration assay.

Enhancement of Teratogenicity

Coadministration of calcium-chelating agents resulted in the enhancement of teratogenicity induced by other chemicals (Wilk, King, and Pratt 1978). The teratogens chlorcyclizine and norchlorocyclizine induced high incidences of cleft palate and skeletal malformations in fetal Sprague-Dawley rats; the chemicals competed with calcium to bind tightly and reversibly to embryonic glycosaminoglycans of cartilage. The test compounds were absorbed onto small pieces of Millipore filters, which were inserted into the uterus onto the intact amniotic sac on GD 15. The test concentrations did not result in maternal toxicity and embryo survival was greater than or equal to 60%. The dams were killed on day 20 and the fetuses were examined for cleft palate and skeletal abnormalities. Study results are shown in Table 14. The authors concluded that the coadministration of a chelating agent increased both the frequency of malformations and the retention of the teratogen.

TABLE 14
Enhancement of teratogenicity (Wilk, King, and Pratt 1978)

Test compound(s)	Fetal survival/sites implanted	Malformations in surviving embryos (%)	
		Cleft palate	Malformed limbs[a]
60 μg norchlorocyclizine	63/79	5	0
60 μg norchlorocyclizine + 20 μg Na_4EDTA	68/111	32[b]	12[c]
60 μg norchlorocyclizine + 20 μg Ca_2EDTA	49/76	2	0
60 μg chlorcyclizine	66/88	0	0
60 μg chlorcyclizine + 20 μg Na_4EDTA	60/94	7[c]	3
20 μg Na_4EDTA	61/83	0	0
20 μg Ca_2EDTA	50/62	0	0
Filter—control	150/188	0	0

[a] All fetuses with malformed limbs had cleft palate as well.
[b] $p \leq .01$ compared to teratogen alone.
[c] $p \leq .05$ compared to teratogen alone.

GENOTOXICITY

In a review of available genotoxicity studies, Heindorff et al. (1983) classified EDTA as a weak mutagen in microbial systems. As shown in Table 15, EDTA and its salts have been evaluated for the potential to cause chromosomal aberrations, semilethals, crossovers, forward mutations, replicative DNA synthesis, DNA strand breaks, dominant lethals, inhibition of metabolic cooperation and contact feeding, and sister-chromatid exchanges with mostly negative results.

EDTA more than doubled the frequency of streptomycin-resistant and phage-producing cells in *Bacillus megatherium* 20Δ. EDTA caused the induction of bacteriophages in *B. megatherium* 20Δ and *Salmonella typhimurium*, but not in lysogenic strains of *E. coli* or *Corynebacterium diphtheriae* (Eisenstark and Kirchner 1956; Northrop 1963, 1968; Kozak and Dobrzański 1970).

In studies using grasshopper germinal cells, murine bone marrow and splenic cells, and human lymphocytes, Disodium EDTA and EDTA caused chromosomal breakage by the chelation of divalent calcium and/or magnesium ions and depleted heavy metal ions from the chromosome (Basrur and Baker 1963; Manna and Das 1971; Saha and Chakrabarty 1973; Saha 1974). Tetrasodium EDTA- and Disodium EDTA-induced cation deficiency did not cause aberrations in Chinese hamster ovary cells or murine germ cells but the chelating agents had effects on mitosis and meiosis (Thompson et al. 1990; Russo and Levis 1992).

EDTA (10^{-3} M) completely dispersed *Drosophila melanogaster* salivary chromosomes into particles ~400 nm in length; chromosomes treated with 0.1 M EDTA appeared normal, and 0.01 M EDTA had an intermediate effect (Mazia 1954). When Kaufmann and McDonald (1957) treated *D. melanogaster* chromosomes with up to 10^{-1} M EDTA, dispersion was not observed. Swelling of nuclei and lateral deformation of the chromosomes were observed, however, after treatment with 10^{-3} M for 2 to 24 hours. Permanent impairment of the integrity of the isolated chromosome was not reported by Cole, Corry, and Langley (1970), who measured the swelling-condensation properties after treatment with 5×10^{-4} M EDTA. Deformation and swelling were reported, as well as other cytological abnormalities (e.g., C-meiotic effects, formation of anaphase and telophase bridges, "sticky" chromatin) in studies using plant chromosomes (Hyde 1956; Kaufmann and McDonald 1957; McDonald and Kaufmann 1957; Hyde and Paliwal 1958).

The increase of meiotic crossovers induced by EDTA depended upon the developmental stage of the test organism, concentration of EDTA, and the chromosome region tested. Conflicting data were reported for studies using green algae and higher plants (Heindorff et al. 1983). EDTA increased the recombination frequency in female *D. melanogaster* larvae (Levine 1955; Steffensen, Anderson, and Kase 1956; Sinkha 1965), but not in adult females (Levine 1955; Steffensen, Anderson, and Kase 1956). In a study by Sinkha (1965), the recombination frequency did not increase when the larvae were exposed to EDTA for the entire developmental period or when 80-hour-old larvae or adults were treated. Disodium EDTA induced aberrations in the salivary chromosomes of *Anopheles stephensi* larvae (Chaudhry, Rani, and Sobti 1988); these aberrations included paracentric heterozygous inversions, deletions, and an unidentified complex aberration. In early *Drosophila* larvae, the increase of crossing-over frequency was dose dependent (Kaufmann, Gay, and McElderly 1957). Full induction occurred at approximately one-half the maximal uptake concentration of EDTA (Colwell and Burdick 1959).

In studies using *Neurospora crassa* and *Zea mays*, certain areas of the chromosomes had decreased crossover frequencies or absences of modification; typically, however, the overall recombination frequencies were concomitantly increased (Steffensen, Anderson, and Kase 1956; Prakash 1963; Ladner 1972; Ihrke and Kronstad 1975; Dishler 1976). EDTA increased the crossover frequency in the euchromatinic region of the chromosome, where there was less spiralization of the chromatids, rather than in the heterochromatinic region (Sinkha 1965). The position of EDTA-induced aberrations were nonrandom; most of the observed break-points were found in the middle and distal regions of the chromosome (Manna 1971).

Mummery et al. (1984) screened EDTA for induction of differentiation in mouse N1E-115 neuroblastoma cells. These investigators reported EDTA as a nonteratogen, giving negative results. The toxic concentration and no effect dose of EDTA were reported as 1×10^{-3} M and 1×10^{-5} M.

The effect of EDTA on mixed-function oxidase activity was evaluated using the liver microsomal assay (LMA) with S9. EDTA (0 to 40 mM) was preincubated with S9 fractions from mice induced with Acrolor 1254 (AC) and and sodium pentabarbital (PB) plus β-naphthoflavone (β-NF). The maximum mean specific activity for aminopyrine (APD) and *p*-nitroanisol-*O*-demethylase (pNAD) from AC-induced mice occurred at 5 mM EDTA and increased 29% and 43%, respectively. A significant inhibition of lipid peroxidation (LP) occurred at 1 mM EDTA in the LMA mixture. Similar results were obtained with S9 fractions from PB + β-NF–induced mice. The LMA was performed with the premutagen cyclophosphamide (CP) using the D7 strain of *Saccharomyces cerevisiae* with and without EDTA. In the presence of EDTA, significant increases occurred in the frequency of mitotic gene conversion, mitotic crossing-over and point reverse mutation induced by CP. These increases are observed when the S9 fractions from PB + β-NF– and AC-induced mice are used (Paolini et al. 1988).

Modification of Genotoxicity

EDTA treatment before or during treatment with γ-rays, β-rays, x-rays, and chemical mutagens often increased the frequency of chromosome aberrations in plant root tip meristems or seeds, although negative results and protective effects were also reported (Heindorff et al. 1983). Calcium Disodium EDTA and

TABLE 15
Genotoxicity of EDTA and its salts

Treatment	Test system	Results	References
	Gene mutations		
EDTA	Phage induction, streptomycin resistance *in Bacillus megatherium* 20Δ and *Escherichia coli*	Increased phage induction; no strep resistance	Northrop 1968
EDTA (0.05%)	Semilethal mutations in *Drosophila melanogaster*	Increased mutations	Baranauskajte, Vasiljauskajte, and Ranchyalis 1972
EDTA	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, ±metabolic activation	No genotoxicity	McCann et al. 1975
Na_2EDTA	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, ±metabolic activation	No genotoxicity	DeFlora 1981
$CaNa_2EDTA$ (0.01–0.8 M)	Rec-assay using *B. subtilis* H17, M45 and *S. typhimurium* TA1978, TA1538	No genotoxicity	Gentile, Hyde, and Schubert 1981
Na_3EDTA	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537, ±metabolic activation	No genotoxicity	Dunkel et al. 1985
Na_3EDTA (60–5000 μg/ml)	Thymidine kinase locus forward mutation in L5178Y mouse lymphoma cells, ±metabolic activation	No increased mutations	McGregor et al. 1988
EDTA, pH ~5.8–6.1	Thymidine kinase locus forward mutation in mouse lymphoma cells, no metabolic activation		Wangenheim and Bolcsfoldi 1988
10.0–25.2 mmol/l		No increased mutations	
30.2 mmol/l		Increase in mutations	
Na_3EDTA	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537, ±metabolic activation	No genotoxicity	Zeiger et al. 1988
Na_2EDTA (5–20 mg/kg in water for 5 days)	Male Swiss albino mice, CFT strain		Muralidhara and Narasimhamurthy 1991
	Bone marrow micronucleus assay	Increased micronuclei	
	Dominant lethals	No increased mutations	
Na_3EDTA	BALB/c-3T3 cells transformation assay	No increase in transformants	Matthews, Spalding, and Tennant 1993
	Chromosome aberrations		
Na_2EDTA	Chromosomal aberrations in *Gesonula punctifrons* germinal cells	No increased aberrations	Ray-Chaudhuri 1961
EDTA (0.05 M)	Chromosome aberrations in mouse bone marrow cells	Increased aberrations	Manna and Das 1971
	Chromosome aberrations in mouse spleen cells	Increased aberrations	Das and Manna 1972
EDTA (10^{-4}–10^{-3} M)	Chromosome aberrations in human leucocytes	Increased aberrations	Basrur and Baker 1963
Na_2EDTA (10^{-3} M, 0.02–0.03 ml, pH ≥ 7.5 via injection)	Chromosomal aberrations in male grasshopper, *Spathosternum prasiniferum* germinal cells	Weak increase	Saha and Chakrabarty 1973; Saha 1974
Na_2EDTA (10 ppm, added to medium)	Chromosomal aberrations in mosquito, *Anopheles stephensi* Liston, larvae salivary chromosomes	Increased aberrations	Chaudhry, Rani, and Sobti 1988
Na_4EDTA (7 mM)	Chromosomal aberrations in Chinese hamster ovary cells	No increased aberrations	Thompson et al. 1990
Na_2EDTA (186 mg/kg, IP)	Chromosome aberrations in BALB/c mouse germ cells	No increased aberrations	Russo and Levis 1992

	DNA damage		
Na_2EDTA (50, 500 μM)	DNA-cell-binding assay in *E. coli*, HeLa, and Ehrlich ascites cells, ±metabolic activation	No genotoxicity	Kubinski, Gutzke, and Kubinski 1981
EDTA	Strand breaks in mouse lymphoma cell DNA (alkaline DNA unwinding and hydroxyapatite elution for DNA strand breaks)		Garberg, Åkerblom, and Bolcsfoldi 1988
10.1–30.4 mmol/l		No increase in breaks	
40.5–50.6 mmol/l		Increase in breaks	
Na_3EDTA (500 mg/kg, 1000 mg/kg by gavage)	In vivo–in vitro replicative DNA synthesis and mean cell viability in $B6C3F_1$ mice	No effect	Miyagawa et al. 1995
	Crossovers/SCEs		
EDTA (added to liquid medium)	Crossovers in *D. melanogaster* larvae	Increase in crossovers	Levine 1955
	Crossovers in *D. melanogaster* adults	No increase in crossovers	Steffensen, Anderson, and Kase 1956
EDTA (10^{-4} M, added to medium for 25 h)	Crossovers in arginine-requiring strains of green algae, *Chlamydomonas reinhardi*	Increase in crossovers	Eversole and Tatum 1956
		No increase in crossovers	Levine and Ebersold 1958
Na_3EDTA (0.01 M, added to liquid media)	Crossovers in *D. melanogaster*		Sinkha 1965
	Larvae—entire development period	No increase in crossovers	
	Larvae—80 h	No increase in crossovers	
	Adults	No increase in crossovers	
K_3EDTA (added to blood)	Human peripheral lymphocytes		Tucker and Christensen 1987
	Sister-chromatid exchanges (SCEs)	Increase in SCEs	
	Cell-cycle kinetics/growth	No effect	
	Other assays		
Na_2EDTA (up to the toxic dose)	Inhibition of metabolic cooperation/contact feeding in Chinese hamster V79 cells, $HGPRT^+$ and $HGPRT^-$	No effect	Umeda, Noda, and Ono 1980
Na_2EDTA (0.2–1.0 mM)	Tradescantia-micronucleus; 15 plant cuttings/group, 6-h exposure in Hoagland's nutrient solution OR gaseous exposure for 6 h	No effect	Ma et al. 1984

other chelating agents reduced the mutagenicity of $Cr_2O_3^{2-}$ in rec-assays using *Bacillus subtilis*, and *S. typhimurium* (Gentile, Hyde, and Schubert 1981). EDTA had a synergistic effect with radiation in the induction of dominant lethals in the female wasp (*Habrobracon juglandis*) after the feeding of 0.09 M EDTA. From days 1 to 7, the incidence of eggs with at least one dominant lethal was 3.4% (control), 26.7% (2500 r), 12.5% (EDTA), and 42.7% (EDTA + 2500 r). The significant increase in mutations, compared to controls, was observed up to the 7th day of treatment, after which the effect was decreased (LaChance 1959). EDTA plus radiation and EDTA plus ethanol, triethylenemelamine, maleic hydrazide, and the mammary gland carcinogen *N*-methyl-*N*-nitrosourea increased the frequency of aberrations in the meiotic cells of *Tradescantia* and *Vicia faba*, respectively (Delone 1958; Michaelis, Nicoloff, and Rieger 1962; Michaelis and Rieger 1965, 1968). Basrur and Baker (1963) reported that the number of chromosomal aberrations was increased significantly after irradiation with x-rays (250 lV, 259 r), compared to treatment with 10^{-4} to 10^{-3} M Sodium EDTA alone.

In other studies (Ondrej 1965, 1970), however, the effect of EDTA plus x-ray irradiation on *Drosophila* was slightly less than the sum of the agents' individual effects and that EDTA did not induce sex-linked recessive lethals. In a study by Sinkha (1965), the crossover frequency did not differ for radiosensitive *D. melanogaster* larvae exposed to EDTA, compared to controls. Only radioresistant larvae had a significantly increased recombination frequency compared to controls with a comparatively small percentage of spontaneous crossing over. The fertility index in radiosensitive larvae was, however, reduced compared to radioresistant larvae. Treatment with both EDTA and the mutagens 1-methyl-2-nitro-1-nitrosoguanidin e and triethylenemelamine did not increase the frequency of aberrations (Gichner, Michaelis, and Rieger 1963). It was concluded that EDTA was not an efficient modifying agent with respect to point mutations induced by irradiation or chemical mutagens (Heindorff et al. 1983).

Effects on DNA Synthesis and Repair

In a number of studies using mammalian test systems, EDTA inhibited DNA synthesis. Inhibition was observed in primary cultures of rabbit adrenal cortex cells after the addition to the medium of 5×10^{-4} M for 60 hours (Lieberman and Ove 1962; Lieberman et al. 1963) or 1.5 μM EDTA for 4 hours (Kishimoto and Lieberman 1964). Inhibition also occurred after infusion of 5×10^{-2} M EDTA for 22 hours into partially hepatectomized rats and the addition of EDTA to human, rat, and pig phytohemagglutinin-stimulated lymphocytes and neonatal rat ventricular cardiac myocytes (Alford 1970; Chesters 1972; Duncan and Dreosti 1975; Kanemaru et al. 1992). Inhibition was not noted in cell lines such as HeLa, L cells, or several-day-old rabbit kidney cultures (Lieberman and Ove 1962). In a study by Krishnamurti, Saryon, and Petering (1980), EDTA at a concentration of 120 nm/mg cell protein (3.02×10^{-6} M) caused a 100-fold decrease of cell numbers in Erhlich ascites cells, relative to controls, and caused significant cell death. The investigators noted that little EDTA accumulated in the cells during either short- or long-term incubations. As a result, EDTA (up to 3 mM) had little, if any, effect on DNA synthesis or thymidine uptake after incubation for 1 hour. Zinc ions were the only cations tested that reversed the effect of the chelating agent, although magnesium enhanced the effect of zinc (Lieberman and Ove 1962; Fujioka and Lieberman 1964).

Inhibition of intestinal DNA synthesis was reported when Long Evans rats were infused IV with 3 to 6 mmol/kg/day Calcium EDTA for 24 hours (Braide and Aronson 1974; Rosenblatt and Aronson 1978). This effect was attributed to the impairment of zinc uptake, as supplementation with the metal protected completely against inhibition and enhanced recovery in rats previously treated with Calcium EDTA alone. Another factor in DNA synthesis inhibition could have been interference with cellular respiration (see "General Biology—Effects on Mucosa"). The mitotic index was similarly decreased when Rosenblatt, Doyle, and Aronson (1978) treated (IV) rats with 6.0 mmol/kg/24 h. In this study, marked morphological changes were also observed, including shortening of the villi, reduced height of the epithelium, and degeneration of the brush border.

The results of these studies suggested that the inhibition of DNA synthesis occurred when replication enzymes were impaired by the chelating action of EDTA. The activities of DNA polymerase and thymidine kinase were inhibited in rabbit hepatocytes, but the activities of dehydrogenases and adenosine deaminase were not affected (Alford 1970; Chesters 1972; Lieberman and Ove 1962; Lieberman et al. 1963; Duncan and Dreosti 1975). The content of diadenosine tetraphosphate decreased in cells treated with EDTA. Zinc chloride stimulated the synthesis of diadenosine tetraphosphate and inhibited its hydrolase. Zinc was considered a putative second messenger of mitogenic induction, hence the observed reversal of DNA synthesis inhibition by the cation (Grummt et al. 1986).

EDTA can interfere with repair of irradiation- or chemical mutagen–induced DNA lesions. The investigators concluded that this interference was due to the direct impairment of repair enzymes or to the modification of chromatin structure (i.e., preventing unwinding of chromatin) such that the accessibility of lesions to repair enzymes was decreased, or to a combination of these two mechanisms (Heindorff et al. 1983).

A concentration of 0.1% EDTA inhibited strongly the induction of premature chromosome condensation (PCC) in hybrid HeLa cells (Rao and Johnson 1971). Induction of PCC resulted from the fusion of a mammalian cell in mitosis with another in interphase. PCC was similar to mitosis and was characterized by the condensation of the chromatin of the interphase nucleus into recognizable chromosomes and the dissolution of the nuclear membrane; however, no mitotic spindle was formed and the chromosomes have no means of transportation to the poles. In this study, the incidence of PCC in binuclear was 39.4%, relative to the control, after the addition of EDTA.

August strain male rats were given a single IP injection of 250 mg/kg folic acid and 0.75 mmol/kg Disodium Calcium EDTA before, at the same time, or after the folic acid injection. Each animal received an IP injection of 250 μCi/kg of [^{3}H]-thymidine. The animals were killed 26 hours after the folate injection. There was a control group and a group of animals that received only folate. The treated groups had a marked reduction in the rate of DNA synthesis in the kidneys 26 hours after folate treatment. However, the rate of DNA synthesis in the intestinal mucosa was not significantly altered (Taylor and Jones 1972).

CARCINOGENICITY

The carcinogenic potential of Trisodium EDTA Trihydrate was assayed during a feeding study using 28-day-old Fischer 344 rats and B6C3F$_1$ mice (NTP 1977). In order to estimate the maximum tolerated dose (MTD), five animals per species per sex per group were fed 4640, 6800, 10,000, 14,700, or 21,600 ppm over a period of 7 weeks, followed by a 7-day observation period. Matched control groups were comprised of 20 animals per sex per species.

In this study, male rats fed $\geq$10,000 ppm and female rats fed $\geq$14,700 ppm had soft stools, which was considered a sign of compound-related toxicity. After the observation period, the body weights of the treated rats (all doses) were comparable to those of the matched controls. No signs of organ toxicity were observed. Male mice fed 21,600 ppm and females fed $\geq$6800 ppm had decreased body weights compared to controls. One male mouse of the high-dose group died. No gross lesions were observed at necropsy.

Based on these results, the low and high concentrations for the chronic feeding study were set at 3750 and 7500 ppm, respectively. The experimental groups were comprised of 50 animals per sex per species, and the matched control groups were comprised of 20 animals per sex per species. The treated rats and mice were fed Trisodium EDTA for 103 weeks, followed by a 1-week observation period. All animals were observed twice daily for signs of toxicity; in addition, they were weighed regularly and palpated for masses at each weighing. Animals appearing moribund were killed for necropsy and collection of tissues for microscopic examination.

No compound-related signs of clinical toxicity were noted. A variety of neoplasms occurred in both treated and control groups of both species, but the two groups were not statistically significantly different. The investigators noted that the lack of appearance of treatment-related neoplasms or other lesions could not be attributed to early mortality, as the survival rate was great for all groups. The investigators concluded that Trisodium EDTA was not carcinogenic to rats or mice under the conditions of this study (NTP 1977).

The elimination of intercellular communication has been correlated to tumor promoting activity. Umeda, Noda, and Ono (1980) investigated the ability of various chemicals to cause inhibition of metabolic cooperation. Wild-type (HGPRT$^+$) Chinese hamster V79 cells were grown in contact with 6-thioguanine–resistant clones (HGPRT$^-$). If contact feeding was inhibited, the resistant phenotype was expressed. Under the conditions of this study, Disodium EDTA did not inhibit metabolic cooperation.

EDTA can enhance 5-aminolevulinic acid (ALA)-induced accumulation of protoporphyrin IX in cells, possibly through the inhibition of ferrochetalase activity and the decrease of heme availability (Brooker et al. 1982; Hanania and Malik 1992; Malik et al. 1995). ALA, a precursor of endogenous porphyrins in the heme synthesis pathway, has been used in conjunction with photoirradiation as a treatment for human basal and squamous cell carcinomas. Female BALB/c mice had C26 colon carcinomas transplanted SC prior to 2 to 6 hours of treatment with topical creams containing 20% ALA, 2% DMSO, and 2% Disodium EDTA (volume = 0.1 ml; shaved skin site area = 1 cm^2). Laser-induced fluorescence analysis indicated that both EDTA and DMSO increased the accumulation of protoporphyrin IX in the skin and implanted carcinomas compared to treatment with ALA alone (Malik et al. 1995).

CLINICAL ASSESSMENT OF SAFETY

Short-Term Studies

When 130 children were treated with chelation therapy (dimercaprol and Calcium Disodium EDTA, 25 mg/kg every 12 hours for 5 days) for asymptomatic lead poisoning, 16% of the children had biochemical evidence of transient nephrotoxicity and 3% had acute oliguric renal failure (Moel and Kumar 1982). Two patients with hypercalcemia who received one or more large doses (6 to 13.5 g over $\sim$1 hour) of Disodium EDTA had renal tubular vacuolization and hydropic degeneration at microscopic examination (Holland, Danielson, and Sahagian-Edwards 1953).

In another study of two hypercalcemic patients, two to seven daily doses (2 to 6 g) of Disodium EDTA resulted in engorgement of tubular cells with eosinophilic granules and the presence of hemorrhages (Dudley et al. 1955).

Studies with [^{14}C]-EDTA were performed in a similar manner to studies with [^{14}C]-Diethylenetriamine Pentaacetic Acid (DTPA). [^{14}C]-DTPA, 10 to 15 mg with a ^{14}C activity of 15 to 20 μCi, was administered IV to 4 patients. Oral doses of [^{14}C]-EDTA, either 3 mg with a ^{14}C activity of 5 to 10 μCi or 50 mg [^{14}C]-EDTA with a ^{14}C activity of 75 to 100 μCi, were administered to two patients. The urinary excretion pattern for [^{14}C]-EDTA was similar to that of [^{14}C]-DTPA. The kidneys were the major route of excretion for [^{14}C]-DTPA after IV injection. At the end of 24 hours, 90% to 100% of the dose of [^{14}C]-DTPA was excreted in the urine. Oral doses of [^{14}C]-DTPA passed through the intestine and 95% to 100% of the dose was recovered in the stool within 2 to 5 days. The urinary excretion was $<$8% in the seven patients who received [^{14}C]-DTPA orally. Results for [^{14}C]-EDTA were similar, although it was administered orally to two patients. Additionally, blood samples taken from 1 hour

to 3 days after oral administration of [^{14}C]-DTPA did not have any ^{14}C activity. Similar results were obtained for [^{14}C]-EDTA (Stevens et al. 1962).

Chronic Studies

Meltzer, Kitchell, and Palmon (1961) treated 81 subjects (normal or with cardiac arrhythmias) with 2000 consecutive infusions of Disodium EDTA over a 2-year period. The chelating agent was administered as a 3-g dose and infused as a 0.5% solution in saline. The test solution was injected over a 2 1/2- to 3-hour period on alternate days, 3 days per week. After the first 20 injections, treatment was discontinued for 6 to 8 weeks. The procedure was then repeated until 2000 injections had been given. Four patients died after receiving 60, 33, 22, and 8 infusions of Disodium EDTA; at necropsy, no evidence of nephrosis was observed. Hypotension, hypocalcemia, burning at the injection site, thrombophlebitis, and GI symptoms were reported by the remaining subjects. The investigators did not observe systemic reactions, histamine-like reactions, anemia, dermatitis, hyperglycemia (or glycosuria), or bone decalcification.

Disodium EDTA was administered IV intermittently in 5-day courses over a 15-month period to four patients with severe, noncalcific acrosclerosis. Each patient received 0.5 g/30 lb body weight dissolved in 500 cc of 5% glucose water and infused slowly over an hour. The total dose ranged from 132 to 212 g. Side effects were minimal and did not require discontinuation of therapy. Eight patients with progressive systemic sclerosis (PSS) served as untreated histologic controls. Five patients served as untreated chemical controls and skin from the forearms of 11 individuals without scleroderma were also chemically analyzed (Keech et al. 1966).

The four patients treated with Disodium EDTA cooperated well and had subjective and objective softening of the skin. At microscopic examination, untreated cases of scleroderma had severe changes. Three of the four patients treated with Disodium EDTA had a return toward normal in their skin. All EDTA cases had definite softening of the skin clinically. Microscopically, this was observed as increased vascularity, return of fixed tissue cells, and the loosening of the collagen bundles in the dermis and the stratum corneum of the epidermis. Normal calcium values from the 11 donors were not statistically significant from the scleroderma average. The magnesium values for the normal donors were 0.19 ± 0.05 μg/mg dry tissue and for the scleroderma group, 0.17 ± 0.05 μg/mg dry tissue.

Skin Irritation and Sensitization

Basketter et al. (1997) investigated the irritancy potential of Disodium EDTA using a 4-hour patch test. A mass of 0.2 g of the powder was applied to the skin of the upper outer arm using a 25-mm Plain Hill Top Chamber containing a moistened Webril pad. To avoid the production of "high reactions," the test material was applied progressively from 15 to 30 minutes to 4 hour. The treatment sites were evaluated at 24, 48, and 72 hours after patch removal for signs of irritancy and graded using a 4-point scale. The positive control was 0.2 ml of 20% sodium dodecyl sulfate. None of the 26 volunteers treated with Disodium EDTA had irritation, compared to 21 of the 26 volunteers treated with SDS.

Meynadier et al. (1982) investigated sensitivity to EDTA 2% in vaseline in 465 individuals (235 men and 230 women) who "most often" suffered from eczema. EDTA caused one positive patch test reaction. No additional details were available.

Raymond and Gross (1969) patch-tested 50 subjects with 0.01% to 10% aqueous Calcium Disodium EDTA (pH 4.6 to 4.8). The test group included both normal volunteers and patients with assorted dermatoses. The test sites were covered with occlusive patches for 48 hours and were examined 30 minutes, 24 hours, and 48 hours after patch removal. One normal volunteer and two patients had positive reactions, and were subsequently tested with 0.1% to 1% EDTA in petrolatum. For the two patients, second challenge was performed "several weeks" after the acute dermatitis subsided. Two of the three subjects also cross-reacted with 0.01% to 10% aqueous ethylenediamine and/or ophthalmic drops containing 0.01% to 0.1% EDTA. The results are presented in Table 16. The investigators concluded that Calcium Disodium EDTA was a weak sensitizer.

Other investigators, however, have stated that EDTA is not a contact sensitizer (Fisher 1979; Rietschel and Fowler 1995). Fisher (1979) reported that no positive reactions were observed when EDTA was included in the North American Contact Dermatitis Group's screening tray for several years, and that EDTA

TABLE 16
Patch test results of Calcium Disodium EDTA (Raymond and Gross 1969)

	Results		
Test compound	Patient 1	Patient 2	Normal subject
0.01% EDTA (aq.)	2+[a]	2+[b]; NT[c]	1+
0.1% EDTA (aq.)	2+	2+; 0	1+
1% EDTA (aq.)	4+	2+; NT	2+
10% EDTA (aq.)	4+	2+; 3+	2+
0.1% EDTA (pet.)	NT	NT; 0	0
1% EDTA (pet.)	NT	NT; 2+	1+
0.01% Ethylenediamine (aq.)	0	2+; NT	0
0.1% Ethylenediamine (aq.)	0	2+; 0	2+
1% Ethylenediamine (aq.)	0	2+; NT	1+
10% Ethylenediamine (aq.)	0	2+; 3+	1+
Visine (0.1% EDTA)	4+	NT; 0	2+
Blephamide (0.01% EDTA)	4+	NT; 0	2+

[a] Reactions observed 48 or 72 h after application: 0, no reactions or transient erythema; 1+, erythema; 2+, erythema and edema; 3+, erythema, papules, and small vesicles; 4+, gross vesiculation; NT, not tested.
[b] During generalized eczematous reaction.
[c] After recovery.

TABLE 17
Products containing Disodiium EDTA that did not cause skin sensitization (CTFA 1998c)

Product	Disodiium EDTA concentration
Moisture rinse	0.1
Maintain shampoo	0.2
Protective spray	0.1
Retain shampoo	0.1
Curl enhancer	0.2
Styling gel	0.02
Intensive conditioner	0.1
Clarifying shampoo	0.2
pH cleaner	0.2
Body wash	0.2
Foaming cleanser	0.2
Eye make-up remover	0.2
Environmental protectant	0.2
Cell recovery	0.2
Eye zone remoisturizer	0.2
Night moisturizing factors	0.2
Matte foundation	0.2
Satin foundation	0.15
Tint	0.2
Face and body foundation	0.2
Vitamin and mineral cleansing mask–1	0.15
Vitamin and mineral cleansing mask–2	0.2
Brow set	0.05
Mascara	0.2
Creme liner	0.2

did not crossreact in 100 patients with ethylenediamine hydrochloride sensitivity.

Data submitted by industry included a list of the results of skin sensitization tests of products with EDTA as an ingredient (Table 17). If products were tested more than once, the formula with the highest concentration of EDTA was reported. The sensitization studies were completed using a repeat-insult patch test (RIPT) protocol using 100 subjects. Most of the products were tested undiluted under occlusive patch conditions and no sensitization was observed (CTFA 1998c).

Immunologic Effects

Two lead workers were injected IV with 20 mg/kg Calcium Disodium EDTA in 250 ml of 5% glucose for 1 h/day, 3 days/week. Worker 1 was treated for 10 weeks, and worker 2 was treated for 6 weeks. Worker 1 had significant increases of the serum concentrations of IgG, IgA, IgM, CD8+, and CD57+ cells. Worker 2 had a significant increase of the serum concentration of IgD, and slight increases of the concentrations of IgG, IgA, and IgM. The concentrations of IgG and CD4+ cells increased gradually in workers 1 and 2, respectively (Sata et al. 1997).

Inhalation Toxicity

Ten patients received a solution of Calcium Disodium EDTA in an aerosol generated by a Drager aerosol unit (particle size not given). The patients had been exposed to an atmospheric concentration of 0.6 to 1.25 mg lead oxide/m^3 while working in a smelting plant. Five patients received 0.6 g Calcium Disodium EDTA daily for 7 days and 2.4 g Calcium Disodium EDTA on the 9th day (group 1). Five patients received four doses of 2.4 g Calcium Disodium EDTA on alternate days (group 2). The excretion of lead in group 1 increased as soon as inhalation therapy started, dropped sharply when it was discontinued, and increased again when treatment resumed. In group 2, the total lead excreted fell steadily and remained at a constant low concentration for 3 days after treatment stopped. Urinary coproporphyrin was reduced in both groups. Blood lead concentrations were reduced by 20% to 45% from the original 0.1 mg/100 ml during treatment in both groups. Stippled cell counts had some irregularity during the course of treatment, but no other changes occurred in the blood analyses. The investigators concluded that individuals vary in the amount of EDTA they absorb, but the value remains constant, between 10% and 30% of the dose administered, for any given individual. However, differences in the excretion of lead between the groups were observed. Group 1 received 6.6 g Calcium Disodium EDTA and excreted an average of 21.6 mg lead, while group 2 received 9.6 g Calcium Disodium EDTA and excreted 14.2 mg lead. The investigators suggest that the difference may be due to the different treatment plans between the two groups (Petrovic et al. 1960).

Horiuchi et al. (1961) selected five male lead workers aged 25 to 49 who had been working in a lead workshop for 3 to 13 years as subjects for an experiment on intratracheal administration of Calcium Disodium EDTA. The working environment of the five subjects had mean values of 0.1 to 1.5 mg/lead/m^3 air. Clinical tests were performed on the subjects 2 weeks before the experiment. The ranges for the clinical analysis were as follows: red blood cell (RBC) count ($\times 10^4$ cm) = 434–479; hemoglobin (g/dl) = 14.5–17.0; stippled cell count/10,000 RBC = 1–22; urine coproporphyrin (mg/l) = 0.25–0.85; blood lead (μg/100 g) = 41.3–104.6, one subject had an undetermined concentration of blood lead; and urine lead (mg/l) = 0.151–0.288).

Approximately 4 g of Calcium Disodium EDTA was administered to each subject for 20 minutes at lunchtime for 4 days. The size of the aerosol particles administered were about 1 micron in diameter. A 20% solution of Calcium Disodium EDTA (5 to 10 ml) was poured into the nebulizers. The volume of lead excreted in the urine increased soon after the administration of Calcium Disodium EDTA and decreased when inhalation was discontinued. Urinary coproporphyrin excretion was markedly decreased during the inhalation period. This continued for at least 2 days after inhalation treatment was discontinued. No

ill-effects of inhalation by this method were found during or after the inhalation period (Horiuchi et al. 1961).

Ashbel, Khil, and Shatrova (1965) studied the effect of aerosolized Calcium Disodium EDTA in 60 workers exposed to lead solder for 1 to 13 years. The patients had mild to moderately severe lead intoxication with lead excretion in the urine of up to 0.1 to 0.22 mg daily. Treatment consisted of aerosol inhalation of Calcium Disodium EDTA twice daily for 7 to 10 days. The dose was 5 ml of a 10% Calcium Disodium EDTA solution so that the total consumed during the treatment was 100 ml of a 10% solution (7 to 10 g). The finely dispersed aerosol of Calcium Disodium EDTA contained particles of 1.5 microns, only about 2% had a larger diameter.

Calcium Disodium EDTA was detected in the blood and urine within 0.5 hours of inhalation, continued to circulate in the blood at concentrations of 1 to 5.5 mg% for 12 to 18 hours and was excreted in the urine for 24 to 30 hours in the amount of 14 to 73 mg. For individual patients, the excretion of Calcium Disodium EDTA through the kidneys ended after 33 to 40 hours.

The patients tolerated the Calcium Disodium EDTA aerosol treatment well and gained a beneficial therapeutic effect. The symptoms of saturnism decreased or discontinued, liver size returned to normal, and the body weight of the patients increased. Hematologic indices improved as a decrease in granular basophilic erythrocytes was observed as well as reticulocytes returned to normal amounts, hemoglobin returned to 80 units from 70 units, and coproporphyrin concentrations in the urine gradually decreased, especially during the days immediately after terminating treatment. The initial amount of lead in the urine was 50 to 140 μg/day and lead excretion increased sharply 2 to 3 days after treatment started and reached 70 to 360 μg. By days 4 to 6, lead excretion averaged 113 μg and continued 2 days after the end of treatment (Ashbel, Khil, and Shatrova 1965).

Calcium Disodium EDTA was administered to four patients in aerosol form. The inhalation dose of Calcium Disodium EDTA was 5 cm^3 of a 10% to 20% solution three times daily. The inhalation procedure was tolerated without pain and no changes in the respiratory organs occurred. The inhalation treatment period was followed by 2 to 3 day pauses and the treatment ended with one or more IV injections. The results of the treatment were considered successful because the porphyrin and "specular" lead in the blood decreased (Teisinger and Srbova 1956).

Teisinger and Srbova (1956) also described the case of a 45-year-old man who worked with lead for 4 years. He had a hemoglobin of 88%, a count of basophil stippled erythrocytes of 3700/million, porphyrin content in the urine of 0.90 mg/24 h, and "specular" lead in the blood of 0.114 mg%. The patient received an inhalation treatment of a 10% to 20% solution of Calcium Disodium EDTA 3 times daily in 5-ml aliquots. The treatment was completed with an IV injection of 1.5 g Calcium Disodium EDTA. The basophil stippled erythrocytes decreased by 700/million, porphyrin decreased to normal values, and "specular" lead in the blood decreased during the 19-day treatment. The patient was released without complaints.

Miscellaneous Effects

The inhibitory effects of EDTA and other chelating agents on nickel-induced hypersensitivity were evaluated by Memon, Molokhia, and Friedmann (1994). A barrier ointment comprised of 15% EDTA (200 mg) in yellow soft paraffin was applied to a 15.7% nickel coin. The ointment inhibited completely the hypersensitivity in 6 of 17 nickel-sensitive subjects and inhibited partially the responses in seven subjects. Intravenous infusions of Disodium EDTA (average dose = 2.8 g; 10% in saline) induced hypocalcemia in patients with cardiac arrhythmias and caused the suppression of ectopic beats. Dipotassium EDTA did not have this effect.

Anghileri (1969) found that increasing concentrations of EDTA increases its binding per milligram of albumin. This binding action increases as the pH values increase from 5.1 to 8.2 and the β-globulin fraction binds more EDTA than other plasma proteins.

Case Reports

A 48-year-old woman with dermatomyositis improved when given IV 2 g of Sodium EDTA dissolved in 500 ml of 5% glucose water over a period of 4 hours, once daily for 6 days. She was treated with corticotropin and hydrocortisone at the same time. A year later her symptoms had returned and another course of Sodium EDTA improved her symptoms (Pollock 1959).

A 40-year-old woman exposed to plutonium was given IV 2.5 g of Calcium Disodium EDTA in 250 cc of saline solution, twice daily for 4 days. Following a 2-day nontreatment period, the treatment was repeated for 12 consecutive days. On the 11th day of treatment, the patient complained of mental dullness, lassitude, nausea, nasal stuffiness, and lower back pain. By day 12, the symptoms became intensified, and the patient also complained of nocturia and frequency, urgency, and burning on urination. At this point, therapy with Calcium Disodium EDTA was discontinued. At urinalysis a 2+ albumin, numerous renal parenchymal cells, many fine, granular casts, and some red and white blood cells were found. The symptoms of renal disturbance disappeared rapidly after treatment with Calcium Disodium EDTA stopped. By the 5th day, the urine was clear and the patient had recovered (Foreman, Finnegan and Lushbaugh 1956).

A 78-year-old woman with recurrent leg ulcers was treated with a topical corticosteroid for mild stasis dermatitis. A weeping dermatitis developed which spread to her legs, arms, and face after applying the topical corticosteroid. After the eruption settled, patch tests were positive on two occasions to Sodium EDTA 1% aq. and pet. (de Groot 1986).

Winder and Curtis (1960) treated 19 patients for acrosclerosis with IV Disodium EDTA given over a 4- to 6-hour period. A majority of the patients received 2.5 to 3.0 g of Disodium EDTA in 500 cc 5% dextrose and water for 15 days. The doses were adjusted for children. Objective improvement occurred in 7/19 patients, 4 were questionably improved and 8/19 did not

improve. Improvement was subjectively determined as healing of gangrenous ulcers, increased joint mobility, decreased pain, softer and more pliable sclerodermatous areas, and decreased dysphagia. One patient developed renal irritation characterized by albuminuria and granular casts that cleared up completely when the drug was discontinued. This was the only patient that received the dose of Disodium EDTA over a 2.5-hour period.

A 47-year-old woman had skin erruptions on the face for 1 year. Physical findings also included elevated skin lesions of the face measuring 0.5 cm × 1.5 cm with a violaceous-to-brown color and irregular borders. Diascopic pressure resulted in a brown-to-apple jelly color. Microscopic examination confirmed the diagnosis of sarcoidosis. She was treated with 3 g of Disodium EDTA IV over a 4-hour period for 5 days. A 2-day nontreatment period followed and the treatment was repeated until 45 g had been given. The skin lesions started to flatten within 11 days after starting therapy. At microscopic examination after 15 days of treatment, lesions included fuzzy, poorly defined epithiloid cells, ragged Langhan's cells, and persistence of lymphocytes. The Disodium EDTA treatment was repeated 2 months later and further improvement was noted (Johnson 1960).

SUMMARY

Ethylenediamine Tetraacetic Acid (EDTA) and its salts are substituted diamines. Hydroxyethyl Ethylenediamine Triacetic Acid (HEDTA) and its salt, Trisodium HEDTA, are substituted amines. EDTA, HEDTA, and their salts function as chelating agents in cosmetics. As of January 1998, Calcium Disodium EDTA, Diammonium EDTA, TEA-EDTA, and HEDTA were not reported used. The remaining ingredients were used in a total of 4168 cosmetic formulations. Data submitted to the FDA in 1984 indicated typical use of EDTA and Tetrasodium EDTA at ≤1% (with one use each at 25%) , Di- and Trisodium EDTA at ≤1% and ≤0.1%, respectively (with a high use up to 5%), and Trisodium HEDTA at ≤1% (with one use at 10%). Dipotassium EDTA and Tripotassium EDTA were used at unknown concentrations. Current concentration of use indicated typical use of EDTA at ≤2%, Disodium EDTA and Trisodium HEDTA at ≤0.5%, Tetrasodium EDTA at ≤0.3% and Trisodium EDTA at ≤0.2%.

Human and rat studies reported that Calcium Disodium EDTA and Disodium EDTA were poorly absorbed via oral routes of administration. A study that dosed human males both IV and IM determined that a majority of the dose of EDTA was excreted entirely through the kidneys by glomerular filtration and tubular excretion. Rats dosed IV, IM, and IP with Calcium C^{14} EDTA elmininated a majority of the chelate in their urine. Most of the chelate was eliminated through the feces after oral administration. A clinical study using male subjects reported almost no absorption of Calcium Disodium EDTA following dermal exposure. EDTA and the edetates can enhance penetration of some other chemicals/substances. Skin irritation and sensitization tests with guinea pigs using Di- and Trisodium EDTA at 0.2 ml and 0.1 ml of a 10% solution, respectively, did not cause any allergic reactions or sensitization.

Under specific in vitro conditions, EDTA and its edetates were cytotoxic to various cell lines. Exposure to EDTA and its edetates over time in rabbits and rats induced morphologic changes in the intestine and inhibited DNA synthesis in the rat intestine. EDTA and its edetates also demonstrated toxicity to the urothelium of the rat and dog.

In all available toxicology studies (rodent and nonrodent species), the lowest dose reported to the FDA that caused toxicological effects was 750 mg/kg/day of either Tetrasodium EDTA, Calcium Disodium EDTA, or Disodium EDTA. The acute LD_{50} in female rats after IP dosing for EDTA and HEDTA was 512.9 mg/kg and 645.1 mg/kg, respectively. The acute oral LD_{50} of Disodium EDTA was ~3.7 mg/kg in male and female rats. Mice dosed IP with Tetrasodium EDTA, Dipotassium EDTA, Calcium EDTA, and Disodium EDTA had LD_{50} values of 350, 450, 500, and 400 mg/kg, respectively. In rats, the LD_{50} value has been reported as 7 mg/kg. Retransfusion with 4.5% isotonic Disodium EDTA at a rate of 10 ml/kg/min in dogs resulted in five of seven dogs dying as a result of increased venous pressure. In a 30-day study, the IP LD_{50} in female rats was 3850 mg/kg for Calcium Disodium EDTA. A single dose of 1 or 2.5 g/kg or daily IP injections of this edetate at 500 mg/kg for 21 days also resulted in mild to severe microscopic changes in the kidneys. Many studies have confirmed the nephrotoxicity of EDTA. Some studies have demonstrated toxic effects, such as increased permeability and severe congestion and hemorrhage, occurring in the intestine. A short-term toxicity study fed Trisodium EDTA Trihydrate to rats and found that male rats fed ≥10,000 ppm and female rats fed ≥14,700 ppm had soft stools, a sign of compound-related toxicity. No signs of organ toxicity or gross pathologic changes were observed. Rats treated for 21 days with Calcium Disodium EDTA died of nephrosis. Administration of EDTA in drinking water to mice at a concentration of 3.4 mM caused changes in the mineral content of certain tissues. Eighty rats fed 5.0% and 10.0% Disodium EDTA for 13 weeks had significantly decreased feed consumption and body weight gain compared to controls. Rats of the high-dose group also had slightly pale livers at necropsy. No other signs of toxicity were observed. Daily IP injections for 6 months of 0.8 to 1 ml of 6% Disodium EDTA in guinea pigs resulted in animals dying of either rupture of the ventricular wall or of right ventricular failure. In several studies rabbits, rats, and dogs were fed Calcium EDTA (high dose 250 mg/kg), Disodium EDTA (high dose 5.0%), and Calcium Disodium EDTA (high doses 250 mg/kg/day and 1.0%). No gross or microscopic abnormalities were observed.

Rats dosed with Disodium EDTA on normal, abraded, and abraded and depilated skin had concentrations of this chelate present in the liver, small intestine, large intestine, and kidney. Various ocular studies have demonstrated that EDTA is both irritating and nonirritating. Other ocular studies have shown that EDTA both increased and decreased the cytoxicity of other

compounds. Calcium EDTA had no effect on the wound healing or breaking strength of skin from rats in short-term studies.

Pregnant rats fed 3% Disodium EDTA at various stages of gestation demonstrated gross malformations when supplemented with 100 ppm zinc as opposed to 1000 ppm zinc. Maternal and fetal toxicity were not observed with ZnEDTA (dosed at 8 and 20 mmol/m^2/day) or ZnCaEDTA (8 mmol/m^2/day). EDTA administered by gavage and in feed to rats on GDs 7 to 14 at 1000 mg/kg/day and 1250 mg/kg/day, respectively, caused maternal toxicity and malformations in the offspring. In mice, however, offspring of dams treated with 1000 mg/kg/day EDTA orally on GDs 8 to 12 demonstrated no malformations. It has been suggested that the zinc deficiency produced by EDTA resulted in teratogenic effects. Treatment of rabbits with a 3% EDTA ophthalmic drop solution resulted in a significant embryocidal effect. Coadministration of Tetrasodium EDTA and Dicalcium EDTA with the teratogens norchlorcyclizine and chlorcyclizine increased the incidence of malformations present in offspring compared to the teratogen alone. Disodium EDTA (380 mg/kg) caused a significant number of resorptions in pregnant rats.

EDTA is classified as a weak mutagen in microbial systems. In a variety of studies using bacteria, mammalian cell lines, and mammals EDTA and its salts gave both positive and negative results. Irradiation of cells prior to exposure to EDTA produced both an increase and a decrease in chromosomal aberrations. In a number of studies using mammalian test systems, EDTA inhibited DNA synthesis. However, in a number of cell lines inhibition was not observed.

Trisodium EDTA Trihydrate was not carcinogenic to rats or mice fed 7500 ppm for 103 weeks. Disodium EDTA does not inhibit metabolic cooperation.

In acute clinical studies, the major route of excretion was the kidneys and intestine after IV and oral administration of EDTA. Exposure to 0.2 g of Disodium EDTA for 4 hours in a human patch test did not produce any signs of reactivity. In various studies, Calcium Disodium EDTA demonstrated either no contact sensitization or weak sensitization. Products that contained Disodium EDTA did not produce sensitization in an RIPT at concentrations of 0.02% to 0.2%. Calcium Disodium EDTA injected IV at 20 mg/kg for 6 and 10 weeks significantly increased serum concentrations of immunoglobulins.

Administration of Calcium Disodium EDTA to patients in an aerosol form produced no ill-effects during and after treatment, as the therapy was well tolerated, with no changes in the respiratory organs observed. Absorption was determined to be 10% to 30% of the dose. Stippled cell counts were somewhat irregular; however, no other changes occurred in the blood analysis as a result of Calcium Disodium EDTA inhalation. Treatment of 130 children with Calcium Disodium EDTA resulted in biochemical evidence of transient nephrotoxicity and acute oliguric renal failure.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel recognized that oral exposures to EDTA produced reproductive/developmental toxicity in test animals. Dermal exposures to EDTA in cosmetic products, however, would result in very little EDTA penetrating the skin, resulting in systemic levels well below those shown to produce adverse effects in the oral dosing studies. The CIR Expert Panel was concerned, however, about EDTA in cosmetic formulations that may be inhaled and absorbed through lung tissue in sufficient amounts to produce adverse systemic effects. An exposure assessment was done assuming the maximum reported historical concentration of EDTA in any cosmetic formulation, 25%. Measurements of the amount of an aerosolized cosmetic formulation available within an individual's personal breathing space demonstrated a maximum concentration of 62 mg/m^3 (Mokler1976; Elder 1983). If a 60-kg individual inhaled 0.5 l of air with the aerosolized cosmetic formulation (assumes only one breath during use of a spray), at the maximum measured concentration that contains EDTA at the maximum concentration of 25%, then the dose of EDTA via inhalation of an aerosolized product would be 1.24×10^{-4} mg/kg. This systemic dose is below that producing reproductive/developmental toxicity. Based on this assessment, the Expert Panel is not concerned about adverse effects of EDTA and its salts in aerosolized formulations.

Very small amounts of the impurity, nitrilotriacetate, can be found in EDTA. Nitrilotriacetate is a potential oral carcinogen in rats at large doses. This is not a concern for human exposure given the low concentrations of use of EDTA and the poor dermal absorption of EDTA. Also, although nitrilotriacetate is readily absorbed from the GI tract of the rat, it is poorly absorbed in man.

The Expert Panel recognized that EDTA and its salts are penetration enhancers of certain compounds because they chelate calcium and, therefore, disrupt intercellular bridging. Formulators should be aware of this when combining EDTA and its salts with those ingredients that have been previously determined to be safe primarily because they were not significantly absorbed.

CONCLUSION

On the basis of the information included in this report, the CIR Expert Panel concludes that EDTA, Calcium Disodium EDTA, Diammonium EDTA, Dipotassium EDTA, Disodium EDTA, TEA-EDTA, Tetrasodium EDTA, Tripotassium EDTA, Trisodium EDTA, HEDTA, and Trisodium HEDTA are safe as used in cosmetic formulations.

REFERENCES

Ahrens, F. A., and A. L. Aronson. 1971. A comparative study of the toxic effects of calcium and chromium chelates of ethylenediaminetetraacetat e in the dog. *Toxicol. Appl. Pharmacol.* 18:10–25.

Aldrich Chemical Company. 1992. *Catalog handbook of fine chemicals*, 587–588. Milwaukee, WI: Aldrich Chemical Company.

Alford, R. H. 1970. Metal cation requirements for phytohemagglutinin-induced transformation of human peripheral blood lymphocytes. *J. Immunol.* 104:698–703.

Altman, J., K. G. Wakim, and R. K. Winkelmann. 1962. Effects of edathamil disodium on the kidney. *J. Invest. Dermat.* 38:215–218.

Anghileri, L. J. 1968. The binding of EDTA to human serum albumin. *Naturwissenschaften* 4:182.

Anghileri, L. J. 1969. Interaction of ethylenediaminetetraacetic acid with plasma proteins. I. The binding of ^{14}C-EDTA to plasma proteins. *Int. J. Clin. Pharmacol.* 2:150–153.

Apgar, J. 1977. Use of EDTA to produce zinc deficiency in the pregnant rat. *J. Nutr.* 107:539–545.

Aronson, A. L., and F. A. Ahrens. 1971. The mechanism of renal transport and excretion of ethylenediaminetetraacetate with interspecies comparisons. *Toxicol. Appl. Pharmacol.* 18:1–9.

Aronson, A. L., and K. M. Rogerson. 1972. Effect of calcium and chromium chelates of ethylenediaminetetraacetate on intestinal permeability and collagen metabolism in the rat. *Toxicol. Appl. Pharmacol.* 21:440–453.

Ashbel, S. I., P. G. Khil, and S. P. Shatrova. 1965. Treatment of occupational saturnism with $CaNa_2$ EDTA aerosols. *Gigiena Truda i Professionsl'nye Zabolevaniia* 9:24–28.

Aungst, B. J., and N. J. Rogers. 1988. Site dependence of absorption-promoting actions of laureth-9, Na salicylate, Na_2EDTA, and aprotinin on rectal, nasal, and buccal insulin delivery. *Pharm. Res.* 5:305–308.

BASF. 1996a. Safety data sheet for Disodium EDTA. Unpublished data submitted by CTFA, 7-17-98. (4 pages.)[2]

BASF. 1996b. Safety data sheet for Disodium EDTA. Unpublished data submitted by CTFA, 7-17-98. (10 pages.)[2]

Baranauskajte, A.-P., O. I. Vasiljauskajte, and V. P. Ranchyalis. 1972. Specific induction of mutations and modification of mutagenic effect of ethylenimine in Drosophila under the influence of ethylenediaminetetraacetate. *Lietuvos TSR Mokslu Akad. Darb. Ser. B (Russ.)* 2:107–113.

Basketter, D. A., M. Chamberlain, H. A. Griffiths, M. Rowson, E. Whittle, and M. York. 1997. The classification of skin irritants by human patch test. *Food Chem. Toxicol.* 35:845–852.

Basrur, V. R., and D. G. Baker. 1963. Human chromosome breakage in low-calcium cultures. *Lancet* 1:1106–1107.

Bauer, R. O., F. R. Rullo, C. Spooner, and E. Woodman. 1952. Acute and subacute toxicity of ethylene diamine tetraacetic acid (EDTA) salts. *Phed. Proc.* 11:321.

Bhattacharyya, D. K., S. Adak, U. Bandyopadhyay, and R. K. Benerjee. 1994. Mechanism of inhibition of horseradish peroxidase-catalysed iodide oxidation by EDTA. *Biochem. J.* 298:281–288.

Borenfreund, E., and C. Shopsis. 1985. Toxicity monitored with a correlated set of cell-culture assays. *Xenobiotica* 15:705–711.

Bourinbaiar, A. S., and C.-H. Lee. 1996. Synergistic effect of gramicidin and EDTA in inhibiting sperm motility and cervical mucus penetration in vitro. *Contraception* 54:367–372.

Braide, V. B. 1984. Calcium EDTA toxicity: Renal excretion of endogenous trace metals and the effect of repletion on collagen degradation in the rat. *Gen. Pharmacol.* 15:37–41.

Braide, V. B. C., and A. L. Aronson. 1974. Calcium ethylenediaminetetraacetate toxicity in the rat: Sequential light- and electron-microscopic studies on chelate-induced enteropathy. *Toxicol. Appl. Pharmacol.* 30:52–62.

Braide, V. B. C., and A. L. Aronson. 1975. Calcium ethylenediaminetetraacetate (CaEDTA) toxicity: Studies on the mechanism of CaEDTA potentiation of pentobarbital anesthesia in the rat. *Toxicol. Appl. Pharmacol.* 32:494–503.

Braide, V. B., V. Grill, and G. Delbello. 1988. Calcium ethylenediaminetetraacetate toxicity in the rat: Ultrastructural effects on skin collagen. *Pharmacol. Res. Commun.* 20:133–146.

Brendel, R., V. Swayne, R. Preston, J. M. Beiler, and G. J. Martin. 1953. Biological effects of salts of ethylenediamine tetra-acetic acid. *J. Am. Pharm. Assoc.* 42:123–124.

Brooker, J. D., G. Strivastava, B. K. May, and W. H. Elliot. 1982. Radiochemical assay for 5-aminolevulinic synthase. *Enzyme* 28:109–119.

Brouwers, J. R. B. J. 1984. Edetaat-chelatietherapie, een behandeling zonder bewijs? *Pharm. Weekbl.* 119:281–284.

Brown, M. R. W., and R. M. E. Richards. 1965. Effect of ethylenediamine tetraacetate on the resistance of *Pseudomonas aeruginosa* to antibacterial agents. *Nature* 207:1391–1393.

Brownie, C. F., C. Brownie, D. Noden, L. Krook, M. Haluska, and A. L. Aronson. 1986. Teratogenic effect of calcium edetate (CaEDTA) in rats and the protective effect of zinc. *Toxicol. Appl. Pharmacol.* 82:426–443.

Budavari, S., ed. 1989. *The Merck index: An encyclopedia of chemicals, drugs, and biologicals*, 11th ed., 550. Rahway, NJ: Merck & Co.

Cantilena, L. R., Jr., and C. D. Klaassen. 1980. The effect of ethylenediaminetetraacetic acid (EDTA) and EDTA plus salicylate on acute cadmium toxicity and distribution. *Toxicol. Appl. Pharmacol.* 53:510–514.

Carlson, M., and L. E. Habeger. 1980. Polarographic determination of edetate disodium in eyewash and ophthalmic decongestant solutions. *J. Pharm. Sci.* 69:826–828.

Chaberek, S., Jr., and A. E. Martell. 1955. Interaction of divalent metal ions with N-hydroxyethylethylenediaminetriacetic acid. *J. Am. Chem. Soc.* 77:1477–1480. (Submitted by FDA in response to FOI request, 1997; 4 pages.)[2]

Chaudhry, S., S. Rani, and R. C. Sobti. 1988. Effect of disodium EDTA on the salivary chromosomes of malaria vector *Anopheles stephensi* Liston (Culicidae:Diptera). *J. Environ. Biol.* 9(3 suppl.):351–356.

Chesters, J. K. 1972. The role of zinc ions in the transformation of lymphocytes by phytohaemagglutinin. *Biochem. J.* 130:133–139.

Cole, A., P. M. Corry, and R. Langley. 1970. Effects of radiation and other agents on the molecular structure and organization of the chromosome. *Genetic Concepts Neoplasia Symp. Fundam. Cancer Res.* 23:346–379.

Colwell, R. R., and A. B. Burdick. 1959. Uptake and effect on crossing-over of ethylenediaminetetraacetic acid (EDTA) in *Drosophila melanogaster*. *Nucleus (Calcutta)* 2:125–130.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1998a. Concentration of use data. Unpublished data submitted by CTFA, 11-18-98. (5 pages.)[2]

CTFA. 1998b. Concentration of use data. Unpublished data submitted by CTFA, 4-13-99. (1 page.)[2]

CTFA. 1998c. Human repeat insult patch testing. Unpublished data submitted by CTFA, 11-5-98. (2 pages.)[2]

CTFA. 1999. Concentration of use data. Unpublished data submitted by CTFA, 4/29/99. (8 pages.)[2]

Dalvi, R. R., C. McGrowan, and A. Ademoyero. 1980. In vivo and in vitro effect of chelating agents on drug metabolizing enzymes of the rat. *Toxicol. Lett.* 6:25–28.

Das, R. K., and G. K. Manna. 1972. Differential chromosomal aberrations produced in the bone marrow and spleen cells of mice treated with two chemicals. *Proc. Indian Sci. Cong.* 59:413–414.

Dagirmanjian, R., E. A. Maynard, and H. C. Hodge. 1956. The effects of calcium disodium ethylenediamine tetraacetate on uranium poisoning in rats. *J. Pharmacol. Exp. Ther.* 117:20–28.

de Groot, A. C. 1986. Contact allergy to EDTA in a topical corticosteroid preparation. *Contact Dermatitis* 15:250–252.

DeFlora, S. 1981. Study of 106 organic and inorganic compounds in the *Salmonella*/microsome test. *Carcinogenesis (Lond.)* 2:283–298.

Delone, N. L. 1958. Breakage of chromosomes of microspores of Tradescantia Paludosa by x-rays and ethylenediamine tetraacedic acid (EDTA). *Biofizika* 2:717–723.

De Paris, P., and S. Caroldi. 1995. In vitro effect of dithiocarbamate pesticides and of $CaNa_2$EDTA on human serum dopamine-β-hydroxylase. *Biomed. Environ. Sci.* 8:114–121.

Desmecht, D. J.-M., A. S. Linden, J.-M. Godeau, and P. M. Lekeux. 1995. Experimental production of hypocalcemia by EDTA infusion in calves: A critical appraisal assessed from the profile of blood chemicals and enzymes. *Comp. Biochem. Physiol.* 110A:115–130.

Dierickx, P. J. 1989. Cytotoxicity testing of 114 compounds by the determination of the protein content in Hep G2 cell cultures. *Toxicol. In Vitro* 3:189–193.

[2]Available for review. Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

Dishler, V. Y. 1976. Induced crossing over and experimental alteration of frequency of genetic recombination in plants; importance of linked genes and genetic recombination in evolution and selection. *Latvijas PSR Zinatnu. Akad. Vestis (Russ.)* 11:54–67.

Done, A. K. 1979. The toxic emergency: Of metals and chelation. *Emerg. Med.* 11:186–189, 195, 201–203, 209, 213, 217–218.

Doolan, P. D., S. L. Schwartz, J. R. Hayes, J. C. Mullen, and N. B. Cummings. 1967. An evaluation of the nephrotoxicity of ethylenediaminetetraacetat e and diethylenetriaminepentaacetat e in the rat. *Toxicol. Appl. Pharmacol.* 10:481–500.

Dow Chemical Company. 1987. Food additive petition on Pentasodium EDTA and Trisodium EDTA. FAP 7B4023. (Submitted by FDA in response to an FOI request, 1997; 6 pages.)[2]

Dudley, H. R., A. C. Ritchie, A. Schilling, and W. H. Baker. 1955. Pathologic changes associated with the use of sodium ethylene diamine tetraacetate in the treatement of hypercalcemia: Report of two cases with autopsy findings. *N. Engl. J. Med.* 255:331–337.

Duncan, J. R., and I. E. Dreosti. 1975. Effect of zinc on DNA and protein synthesis in dividing rat lymphocytes. *Agrochemophysica* 7:1–4.

Dunkel, V., E. Zeiger, S. Haworth et al. 1985. Reproducibility of microbial mutagenicity assays. II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ. Mutagen* 7:1–248.

Eagon, R. G., and K. J. Carson. 1965. Lysis of cell walls and intact cells of *Pseudomonas aeruginosa* by ethylenediamine tetraacetic acid and by lysozyme. *Can. J. Microbiol.* 11:193–201.

Eisenstark, A., and C. Kirchner. 1956. Genetic effects of chelation on phage-bacterial systems and on bacteria alone. *Genetics* 41:640–641.

Elder, R. L. 1983. Final report on the safety assessment of vinyl acetate/crotonic acid polymer. *JACT* 2(5):125–140.

Eversole, R. A., and E. L. Tatum. 1956. Chemical alteration of crossing-over frequency in *Chlamydomonas*. *Proc. Natl. Acad. Sci. U.S.A.* 42:68–73.

Feldman, S., and M. Gibaldi. 1969. Physiologic surface-active agents and drug absorption. II: Comparison of the effect of sodium taurodeoxycholat e and ethylenediaminetetraaceti c acid on salicylamide and salicylate transfer across the everted rat small intestine. *J. Pharm. Sci.* 58:967–970.

Fisher, A. A. 1979. Cross-reactions between ethylenediamine tetraacetate and ethylenediamine hydrochloride. *J. Am. Acad. Dermatol.* 1:560.

Flint, O. P., T. C. Orton, and R. A. Ferguson. 1984. Differentiation of rat embryo cells in culture: Response following acute maternal exposure to teratogens and non-teratogens. *J. Appl. Pharmacol.* 4:109–116.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA database*. Washington, DC: FDA.

FDA. 1998a. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

FDA. 1998b. Food additive safety profiles of Tetrasodium EDTA, Disodium EDTA, Calcium Disodium EDTA, and Dipotassium EDTA. *FDA database*. Washington, DC: FDA.

Foreman, H., C. Finnegan, and C. C. Lushbaugh. 1956. Nephrotoxic hazard from uncontrolled edathamil calcium-disodium therapy. *JAMA* 160:1042–1046.

Foreman, H., M. Vier, and M. Magee. 1953. The metabolism of C14-labeled ethylenediaminetetraaceti c acid in the rat. *J. Biol. Chem.* 203:1045–1053.

Foreman, H. F., and T. T. Trujillo. 1954. The metabolism of C^{14} labeled ethylenediaminetetraaceti c acid in human beings. *J. Lab. Clin. Med.* 43:566–567.

Forland, M., T. N. Pullman, A. R. Lavender, and I. Aho. 1966. The renal excretion of ethylene diamineteraacetat e in the dog. *J. Pharmacol. Exp. Ther.* 153:142–147.

Fregert, S., and B. Gruvberger. 1980. Formaldehyde in technical EDTA and NTA. *Contact Dermatitis* 6:366.

Fujioka, M., and J. Lieberman. 1964. A Zn^{++} requirement for synthesis of deoxyribonucleic acid by rat liver. *J. Biol. Chem.* 239:1164–1167.

Furlani, A., and R. Vertua. 1970. Tissue radioactivity after skin application of labeled ethylenediaminetetraaceti c acid in the rat. *Pharmacol. Res. Commun.* 2:77–81.

Garberg, P., E.-L. Åkerblom, and G. Bolcsfoldi. 1988. Evaluation of a genotoxicitiy test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. *Mutat. Res.* 203:155–176.

Gasset, A. R., and T. Akaboshi. 1977. Embryopathic effect of ophthalmic EDTA. *Invest. Ophthalmol. Visual Sci.* 16:652–654.

Gennaro, A. R., ed. 1990. *Remington's pharmaceutical sciences*, 18th ed., 824–825, 1321. Easton, PA: Mack Publishing Co.

Gentile, J. M., K. Hyde, and J. Schubert. 1981. Chromium genotoxicity as influenced by complexation and rate effects. *Toxicol. Lett.* 7:439–448.

Gichner, T., A. Michaelis, and R. Rieger. 1963. Radiomimetic effects of 1-methyl-2-nitrosoguanidine in *Vicia faba*. *Biochem. Biophys. Res. Commun.* 11:120–124.

Glavin, G. B., and S. Szabo. 1993. Effects of the Ca^{2+} chelators EGTA and EDTA on ethanol- or stress-induced gastric mucosal lesions and gastric secretion. *Eur. J. Pharmacol.* 233:269–273.

Gogate, M. G., G. V. Joglekar, V. G. Ganla, and J. H. Balawani. 1965. Effect of disodium ethylenediamine tetra-acetic acid in normal dogs on peripheral utilisation of glucose. *J. Exp. Med. Sci.* 9:16–19.

Gosselin, R. E., R. P. Smith, and H. C. Hodge. 1984. *Clinical toxicology of commercial products*, 5th ed., II-381, III-163–166. Baltimore, MD: Williams & Wilkins.

Grant, J., ed. 1972. *Hackh's chemical dictionary*, 4th ed., 230, 234, 254. New York, NY: McGraw-Hill Book Co.

Grant, W. 1952. New treatment for calcific corneal opacities. *AMA Arch. Ophthal.* 48:681–685.

Grass, G. M., R. W. Wood, and J. R. Robinson. 1985. Effects of calcium chelating agents on corneal permeability. *Invest. Ophthalmol. Visual Sci.* 26:110–113.

Gray, L. E., and R. J. Kavlock. 1984. An extended evaluation of an in vivo teratology screen utilizing postnatal growth and viability in the mouse. *Teratog. Carcinog. Mutagen* 4:403–426.

Greiff, L., P. Wollmer, M. Andersson, and C. G. A. Persoon. 1994. Human nasal absorption of ^{51}Cr-EDTA in smokers and control subjects. *Clin. Exp. Allergy* 24:1036–1040.

Grier, M. T., and D. G. Meyers. 1993. So much writing, so little science: A review of 37 years of literature on edetate sodium chelation therapy. *Ann. Pharmacother.* 27:1504–1509.

Grummt, D., C. Weinmann-Dorsch, J. Schneider-Schaulies, and A. Lux. 1986. Zinc as a second messenger of mitogenic induction: Effects on diadenosine tetraphosphate (Ap_4A) and DNA synthesis. *Exp. Cell Res.* 163:191–200.

Hall, L., and L. Takahashi. 1988. Quantitative determination of disodium edetate in ophthalmic and contact lens care solutions by reversed-phase high-performance liquid chromatograph y. *J. Pharm. Sci.* 77:247–250.

Hamano, T., Y. Mitsuhashi, K. Tanaka et al. 1985. A separative determination of EDTA-2Na and EDTA-metals in foods by column chromatography. *J. Food Hyg. Soc. Jpn.* 26:630–637.

Hanania, J., and Z. Malik. 1992. The effect of EDTA and serum on endogenous-porphyrin accumulation and photodynamic sensitization of human K562 leukemic cells. *Cancer Lett.* 65:127–131.

Harmony, R., A. Fernández-Bouzas, A. Toro, and S. Szava. 1973. The epileptogenic effects of Na_2EDTA topical treatment of rat cerebral cortex. *Physiol. Bohemoslav. (Engl. Ed. Cesk. Fysiol.)* 22:297–303.

Hart, J. R. 1984. Chelating agents as preservative potentiators. In *Cosmetic and drug preservation principles and practice*, ed. J. J. Kabara, 323–337. New York, NY: Marcel Dekker.

Hasegawa, R., Y. Nakaji, Y. Kurokawa, and M. Tobe. 1989. Acute toxicity tests on 113 environmental chemicals. *Sci. Rep. Res. Inst. Tohoku Univ. C* 36:10–16.

Hazardous Substances Data Base (HSDB). 1998. Entries on EDTA, Calcium Disodium EDTA, Tetrasodium EDTA, Trisodium EDTA, HEDTA, and Trisodium HEDTA. Computer printout from the HSDB. Bethesda, MD: National Library of Medicine (NLM).

Heindorff, K., O. Aurich, A. Michaelis, and R. Rieger. 1983. Genetic toxicity of ethylenediaminetetraaceti c acid (EDTA). *Mutat. Res.* 115:149–173.

Henck, J. W., D. D. Lockwood, and K. J. Olson. 1980. Skin sensitization potential of trisodium ethylenediaminetetraacetate. *Drug Chem. Toxicol.* 3:99–103.

Holland, J. F., E. Danielson, and A. Sahagian-Edwards. 1953. Use of ethylene diamine tetraacetic acid in hypercalcemic patients. *Proc. Soc. Exp. Biol. Med.* 84:359–364.

Horiuchi, K., Y. Masuya, K. Hashimoto, I. Asano, R. Iwamoto, Y. Komoike, and K. Aratake. 1961. An experience of intratracheal administration of calcium disodium ehtylene-diamine-tetraacetate (Ca-EDTA) by lead workers at their actual working place. *Osaka City Med. J.* 7:59–62.

Hugenschmidt, S., F. Planas-Bohne, and D. M. Taylor. 1993. On the toxicity of low doses of tetrasodium-ethylenediamine-tetraacetat e (Na-EDTA) in normal rat kidney (NRK) cells in culture. *Arch. Toxicol.* 67:76–78.

Hyde, B. B. 1956. The effect of Versene on the structure of plant chromosomes. *Genetics* 41:648.

Hyde, B. B., and R. L. Paliwal. 1958. Studies on the role of cations in the structure and behavior of plant chromosomes. *Am. J. Bot.* 45:433–438.

Ihrke, C. A., and W. E. Kronstad. 1975. Genetic recombination in maize as affected by ethylenediaminetetraaceti c acid and dimethyl sulfoxide. *Crop Sci.* 15:429–431.

J. Lowenstein and Sons, Inc. 1996. Specifications of Disodium EDTA. Unpublished data submitted by CTFA, 7-17-98. (1 page.)[2]

Jarvis, M. F., and G. W. Gessner. 1994. Dithiothreitol, sodium chloride, and ethylenediaminetetraaceti c acid increase the binding affinity of [^{125}I]angiotensin IV to AT_4 receptors in bovine adrenal cortex. *Peptides* 15:1037–1044.

Johnson, S. 1960. Use of the chelating agent edathamil disodium in acrosclerosis, sarcoidosis and other skin conditions with comments on tryptophan metabolism in sarcoidosis. *Wisonsin Med. J.* 59:651–656.

Kabara, J. J. 1980. GRAS antimicrobial agents for cosmetic products. *J. Soc. Cosmet. Chem.* 31:1–10.

Kanemaru, Y., M. J. Rossowska, C. H. Narayanan, and T. Nakamoto. 1992. Effect of caffeine and zinc on DNA and protein synthesis of neonatal rat cardiac muscle cell in culture. *Res. Exp. Med.* 192:115–122.

Kaufmann, B. P., H. Gay, and M. J. McElderry. 1957. Effect of ribonuclease on crossing over in Drosophila. *Proc. Natl. Acad. Sci. U.S.A.* 43:255–261.

Kaufmann, B. P., and M. R. McDonald. 1957. The nature of the changes effected in chromosomal materials by the chelating agent EDTA. *Proc. Natl. Acad. Sci. U.S.A.* 43:262–270.

Kawamata, K., H. Yoshimoto, J. Momma et al. 1980. Comparative toxicity studies of Na_2EDTA and $CaNa_2$EDTA in rats. *Jpn. J. Pharmacol.* 30(suppl.):234P.

Keech, M. K., D. S. McCann, A. J. Boyle, and H. Pinkus. 1966. Effect of ethylenediaminetetraaceti c acid (EDTA) and tetrahydroxyquinon e on sclerodermatous skin. *J. Invest. Dermat.* 47:235–246.

Kimmel, C. A. 1975. Fetal gonad dysgenesis following EDTA administration. *Teratology* 11:26A.

Kimmel, C. A. 1977. Effect of route of administration on the toxicity and teratogenicity of EDTA in the rat. *Toxicol. Appl. Pharmacol.* 40:299–306.

Kimmel, C. A., and C. S. Sloan. 1975. Studies on the mechanism of EDTA teratogenesis. *Teratology* 12:330–331.

Kishimoto, S., and I. Lieberman. 1964. Synthesis of RNA and protein required for the mitosis of mammalian cells. *Exp. Cell Res.* 36:92–101.

Koshakji, R. P., and A. R. Schulert. 1973. Biochemical mechanisms of salicylate teratology in the rat. *Biochem. Pharmacol.* 22:407–416.

Kozak, W., and W. T. Dobrzański. 1970. Activity of some agents in the lysogenic induction of *Corynebacterium diphtheriae* strain no. 25. *Bull. Acad. Pol. Sci. Ser. Sci. Biol.* 18:445–450.

Kozlov, A. B., E. A. Ostrachovitch, and I. B. Afanas'ev. 1994. Mechanism of inhibitory effects of chelating drugs on lipid peroxidation in rat brain homogenates. *Biochem. Pharmacol.* 47:795–799.

Krari, N., and P. Allain. 1991. Effects of three chelating agents, EDTA, NTA, and TPP, on the concentration of elements in rat tissues. *Biol. Trace Elem. Res.* 29:125–131.

Krishnamurti, C., L. A. Saryon, and D. H. Petering. 1980. Effects of ethylenediaminetetraacetic acid and 1,10-phenanthrolin e on cell proliferation and DNA synthesis of Ehrlich ascites cells. *Cancer Res.* 40:4092–4099.

Kubinski, H., G. E. Gutzke, and Z. O. Kubinski. 1981. DNA-cell-binding (DCB) assay for suspected carcinogens and mutagens. *Mutat. Res.* 89:95–136.

LaChance, L. E. 1959. The effect of chelation and X-rays on fecundity and induced dominant lethals in *Habrobracon. Radiat. Res.* 11:218–228.

Ladner, L. 1972. A possible causal relationship between iron deficiency, inhibition of DNA synthesis, and reduction of meiotic recombination frequency in *Neurospora crassa*. *Mol. Gen. Genet.* 119:103–117.

Lee, Y. K., and S. J. Pirt. 1979. Instability and toxic effects of ethylenediamine tetraacetic acid-containing culture media when exposed to sunlight. *FEMS Microbiol. Lett.* 6:379–381.

Levine, R. P. 1955. Chromosome structure and the mechanism of crossing over. *Proc. Natl. Acad. Sci. U.S.A.* 41:727–730.

Levine, R. P., and W. T. Ebersold. 1958. The relation of calcium and magnesium to crossing-over in *Chlamydomonas reinhardi*. *Z. Vererbungsl.* 89:631–635.

Lewis, R.J., Sr. 1993. *Hawley's condensed chemical dictionary*, 12th ed., 486–487, 621. New York, NY: Van Nostrand Reinhold.

Lieberman, J., R. Abrams, N. Hunt, and P. Ove. 1963. Levels of enzyme activity and deoxyribonucleic acid synthesis in mammalian cells cutured from the animal. *J. Biol. Chem.* 238:3955–3962.

Lieberman, J., and P. Ove. 1962. Deoxyribonucleic acid synthesis and its inhibition in mammalian cells cultured from the animal. *J. Biol. Chem.* 237:1634–1642.

Ma, T., M. M. Harris, V. Anderson, I. Ahmed, K. Mohammad, J. L. Bare, and G. Lin. 1984. Tradescantia-micronucleus (Trad-MCN) tests on 140 health-related agents. *Mutat. Res.* 138:157–167.

Magee, R. 1985. Chelation therapy of atherosclerosis. *Med. J. Aust.* 142:514–515.

Malik, Z., G. Kostenich, L. Roitman, B. Ehrenberg, and A. Orenstein. 1995. Topical application of 5-aminolevulinic acid, DMSO, and EDTA: Protoporphyrin IX accumulation in skin and tumours of mice. *J. Photochem. Photobiol. B: Biol.* 28:213–218.

Manna, G. K. 1971. Bone marrow chromosome aberrations in mice induced by physical, chemical, and living mutagens. *J. Cytol. Genet. (India)* Cong. Suppl. 144–150.

Manna, G. K., and D. K. Das. 1971. A study on the conjoint effects of two chemicals, ethylenediamine tetraacetic acid (EDTA) and maleic hydrazide (MH) on the bone marrow chromosomes of mice. *Proc. Ind. Sci. Cong.* 58:635.

Matthews, E. J., J. W. Spalding, and R. W. Tennant. 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 Perspect.* 101(suppl. 2):347–482.

Mazia, D. 1954. The particulate organization of the chromosome. *Proc. Natl. Acad. Sci. U.S.A.* 40:521–527.

McCann, J., E. Choi, E. Yamazaki, and B. N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci. U.S.A.* 72:5135–5139.

McClain, R. M., and J. J. Siekierka. 1975. The placental transfer of lead-chelate complexes in the rat. *Toxicol. Appl. Pharmacol.* 31:443–451.

McDonald, M. R., and B. P. Kaufmann. 1957. Production of mitotic abnormalities by ehtylenediaminetetraaceti c acid. *Exp. Cell Res.* 12:415–417.

McGregor, D. B., A. Brown, P. Cattanach et al. 1988. Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ. Mol. Mutagen* 12:85–54.

McKay, D. M., J. R. Ramage, K. Rangachari, and M. H. Perdue. 1994. Effect of region, temperature, and neuronal blockade on sodium and ^{51}Cr-EDTA across canine gastrointestinal mucosae *in vitro*. *Comp. Biochem. Physiol.* 107A:711–717.

Meltzer, L. E., J. R. Kitchell, and F. Palmon. 1961. The long term use, side effects, and toxicity of disodium ethylenediamine tetraacetic acid (EDTA). *Am. J. Med.* 242:11–17.

Memon, A. A., M. M. Molokhia, and P. S. Friedmann. 1994. The inhibitory effects of topical chelating agents and antioxidants on nickel-induced hypersensitivity reactions. *J. Am. Acad. Dermatol.* 30:560–565.

Meyer, D. R., and J. P. McCulley. 1991. Differential effect of chelation on the pH tolerance of corneal epithelium in tissue culture. *Ophthal. Res.* 23:204–212.

Meynadier, J. M., J. Meynadier, A. Colmas et al. 1982. Preservative sensitivity. *Ann. Dermatol. Venereol.* 109:1017–1023.

Michaelis, A., H. Nicoloff, and R. Rieger. 1962. Influences of EDTA on the induction of chromatid aberrations by triethylenemelamine and ethyl alcohol. *Biochem. Biophys. Res. Commun.* 9:280–283.

Michaelis, A., and R. Rieger. 1965. Chelate formation as a sensitizing factor in the production of chromatid aberrations by radiomimetic agents. *Induction Mutat. Mutat. Process Proc. Symp.* 101–106.

Michaelis, A., and R. Rieger. 1968. On the distribution between chromosomes of chemically induced chromatid aberrations: Studies with a new karyotype of *Vicia faba*. *Mutat. Res.* 6:81–92.

Misra, M., M. Athar, S. K. Hasan, and R. C. Srivastava. 1988. Alleviation of nickel-induced biochemical alterations by chelating agents. *Fundam. Appl. Toxicol.* 11:285–292.

Mitchell, I., and P. J. Gilbert. 1984. The effect of pretreatment of *Escherichia coli* CM891 with ethylenediaminetetraacetate on sensitivity to a variety of standard mutagens. *Mutat. Res.* 140:13–19.

Miyagawa, M., H. Takahashi, A. Sugiyama et al. 1995. The in vivo-in vitro replicative DNA synthesis (RDS) test with hepatocytes prepared from male B6C3F1 mice as an early prediction assay for putative nongenotoxic (Ames-negative) mouse hepatocarcinogens. *Mutat. Res.* 343:157–183.

Moel, D. I., and K. Kumar. 1982. 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* 70:259–262.

Mokler, B. V. 1976. Characterization of respirable particles generated during the use of aerosolized consumer products. Annual report, pp. 309–315. Albuquerque, NM: Inhalation Toxicology Research Institute.

Monteiro, M. C. A., G. Alonso, H. Ajzen, and A. B. Pereira. 1994. Assessment of glomerular filtration rate utilizing subcutaneously injected ^{51}Cr-EDTA. *Braz. J. Med. Biol. Res.* 27:2557–2564.

Müller, M., and A. Haeberli. 1994. pH-Dependent formation of ethylenediaminetetraacetic acid supramolecular aggregates. *FEBS Lett.* 340:17–21.

Mummery, C. L., C. E. Van Den Brink, P. T. Van Der Sagg, and S. W. De Laat. 1984. A short-term screening test for teratogens using differentiating neuroblastoma cells in vitro. *Teratology* 29:271–279.

Murakami, T., M. Misaki, Y. Kojima et al. 1993. Effect of absorption promoters on subcutaneous absorption of human epidermal growth factor in rats. *J. Pharm. Sci.* 82:236–239.

Muralidhara, K., and K. Narasimhamurthy. 1991. Assessment of *in vivo* mutagenic potency of ethylenediaminetetraacetic acid in albino mice. *Food Chem. Toxicol.* 29:845–849.

National Academy of Sciences (NAS). 1996. *Food chemicals codex*, 4th ed., 59, 125–126, 856–857. Washington, DC: National Academy Press.

National Toxicology Program (NTP). 1977. Bioassay of Trisodium Ethylenediaminetetraacetate Trihydrate (EDTA) for possible carcinogenicity (CAS No. 150-38-9). Technical Report 11. NTIS Order No. PB270938/AS.

NTP. 1998. Entry on EDTA. Computer printout from the NTP Health and Safety Database. Betheda, MD: National Institutes of Health. (http://ntp-server.niehs.nih.gov/htdocs/Results_status/Resstatt/C03974.Html)

Nikitakis, J. M., and G. N. McEwen, Jr., eds. 1990a. *CTFA compendium of cosmetic ingredient composition—descriptions I and II.* Washington, DC: CTFA.

Nikitakis, J. M., G. N. McEwen, Jr., eds. 1990b. *CTFA compendium of cosmetic ingredient composition—specifications*. Washington, DC: CTFA.

Nishihata, T., C.-S. Lee, H. Rytting, and T. Higuchi. 1987. The synergistic effects of concurrent administration to rats of EDTA and sodium salicylate on the rectal absorption of sodium cefoxitin and the effects of inhibitors. *J. Pharm. Pharmacol.* 39:180–184.

Nishihata, T., H. Tomida, G. Frederick, H. Rytting, and T. Higuchi. 1985. Comparison of the effects of sodium salicylate, disodium ethylenediaminetetraacetic acid and polyoxyethylene-2,3-lauryl ether as adjuvants for the rectal absorption of sodium cefoxitin. *J. Pharm. Pharmacol.* 37:159–163.

Northrop, J. H. 1963. Studies on the origin of bacterial viruses. VII. The effect of various mutagens (urethane, ethyl urethane, hydrogen peroxide, desoxycholate, maleic hydrazide, butadiene dioxide, triethylene melamine, Versene, and acriflavine) on the proportion of virus-producing and streptomycin-resistant cells in cultures of *B. megatherium* 20Δ. *J. Gen. Physiol.* 46:971–981.

Northrop, J. H. 1968. Appearance of virulent bacteriophages in lysogenic *E. coli* cultures after prolonged growth in the presence of triethylenemelamine. *J. Gen. Physiol.* 52:136–143.

Ondřej, M. 1965. Influence of interaction between EDTA and X-rays on mutation and crossover frequencies in *Drosophila melanogaster*. *Induction Mutat. Mutat. Process Proc. Symp.* 26–29.

Ondřej, M. 1970. Genetic effects of EDTA alone and in combination with radiation. *Dros. Info. Serv.* 45:69–70.

Oosterlinck, W., R. Verbeeck, C. Cuvelier, and D. Vergauwe. 1992. Rationale for local toxicity of calcium chelators. *Urol. Res.* 20:19–21.

Oosterlinck, W., R. Verbeeck, C. Cuvelier, H. Verplaetse, and A. Verbaeys. 1991. Toxicity of litholytic ethylenediaminetetraacetic acid and solutions to the urothelium of the rat and the dog. *Urol. Res.* 19:265–268.

Oser, B. L., M. Oser, and H. C. Spencer. 1963. Safety evaluation studies of calcium EDTA. *Toxicol. Appl. Pharmacol.* 5:142–162.

Paolini, M., F. Tonelli, C. Bauer, G. Bronzetti, C. Corsi, and G. Cantelli-Forti. 1988. EDTA affects cytochrome P450-dependent biotransformation reactions during incubations for the liver microsomal assay. *Mutat. Res.* 208:189–194.

Payne, J. M., and B. F. Sansom. 1964. The relative toxicity in rats of disodium ehtylene diamine tetra-acetate, sodium oxalate and sodium citrate. *J. Physiol.* 170:613–620.

Peart, W. S., T. Quesada, and I. Tenyi. 1977. The effects of EDTA and EGTA on renin secretion. *Br. J. Pharmacol.* 59:247–252.

Perry, H. M., and E. F. Perry. 1959. Normal concentrations of some trace metals in human urine: Changes produced by ethylenediaminetetraacetate. *J. Clin. Invest.* 38:1452–1463.

Petrović, L. J., M. Stanković, M. Savićević, and D. Poleti. 1960. Aerosol inhalation of CaNa2EDTA (Mosatil) by workers constantly exposed to lead poisoning. *Br. J. Industr. Med.* 17:201–204.

Piepsz, A., H. Pintelon, and H. R. Ham. 1994. Estimation of normal chromium-51 ethylene diamine tetra-acetic acid clearance in children. *Eur. J. Nucl. Med.* 21:12–16.

Pillai, S. 1982. Binding of ethylene diamine tetra acetate to erythrocyte cell membranes. *Indian J. Med. Res.* 76:643–648.

Pollock, R. 1959. Dermatomyositis treated with sodium ethylenediamine-tetra-acetate. *California Med.* 90:284–287.

Prakash, V. 1963. Effects of chelating agents on crossing-over in *Neurospora crassa*. *Genetica* 34:121–151.

Praus, R., I. Brettschneider, and L. Krejčí. 1976. Ethylenediamine tetraacetate: Its release from hydrophilic gel contact lenses, intraocular penetration and effect on calcium in the cornea after lime burns. *Ophthal. Res.* 8:161–168.

Rao, P. N., and R. T. Johnson. 1971. Mammalian cell fusion IV: Regulation of chromosome formation from interphase nuclei by various chemical compounds. *J. Cell Physiol.* 78:217–224.

Ray-Chaudhuri, S. P. 1961. Induction of chromosome aberrations in the spermatocytes of grasshoppers. *Nucleus (Calcutta)* 4:47–66.

Raymond, J. Z., and P. R. Gross. 1969. EDTA: Preservative dermatitis. *Arch. Dermatol.* 100:436–440.

Registry of Toxic Effects of Chemical Substances (RTECS). 1997. Entries on EDTA, Calcium Disodium EDTA, Disodium EDTA, Tetrasodium EDTA, Tripotassium EDTA, Trisodium EDTA, HEDTA, and Trisodium HEDTA. Computer printout from the RTECS Database. Bethesda, MD: National Library of Medicine.

Reuber, M. D. 1967a. Severe nephrosis in older male rats given calcium disodium edetate. *Arch. Environ. Health* 15:141–146.

Reuber, M. D. 1967b. Hepatic lesions in young rats given calcium disodium edetate. *Toxicol. Appl. Pharmacol.* 11:321–326.

Reuber, M. D. 1969. Calcium disodium edetate nephrosis in female rats of varying ages. *J. Pathol.* 97:335–338.

Reuber, M. D., and C. W. Lee. 1966. Calcium disodium edetate nephrosis in inbred rats. *Arch. Environ. Health* 13:554–557.

Reuber, M. D., and G. C. Schmieler. 1962. Edetate kidney lesions in rats. *Arch. Environ. Health* 5:430–436.

Rietschel, R. L., and J. F. Fowler, Jr., eds. 1995. *Fisher's contact dermatitis*, 4th ed., 227, 341–342, Baltimore, MD: Williams & Wilkins. 287–288, 684–685, 1003t.

Rosenblatt, D. E., and A. L. Aronson. 1978. Calcium ethylenediaminetetraacetate (CaEDTA) toxicity: Time- and dose-response studies on intestinal DNA synthesis in the rat. *Exp. Mol. Pathol*. 28:202–214.

Rosenblatt, D. E., D. G. Doyle, and A. L. Aronson. 1978. Calcium ethylenediaminetetraacetate (CaEDTA) toxicity: Time- and dose-response studies on intestinal morphology in the rat. *Exp. Mol. Pathol*. 28:215–226.

Russo, A., and A. G. Levis. 1992. Further evidence for the aneuoploidogenic properties of chelating agents: Induction of micronuclei in mouse male germ cells by EDTA. *Environ. Mol. Mutagen* 19:125–131.

Ryo, R., W. Sugano, M. Goto et al. 1994. Platelet release reaction during EDTA-induced platelet agglutinations and inhibition of EDTA-induced platelet agglutination by anti-glycoprotein IIb/IIIa complex monoclonal antibody. *Thromb. Res.* 74:265–272.

Saha, A. K. 1974. EDTA-induced chromosome aberrations in grasshopper germinal cells. *Chromosome Inform. Serv.* 16:18–20.

Saha, A. K., and A. Chakrabarty. 1973. Cytological effect of versene on the production of dicentric bridges in spermatocytic chromosomes of grasshopper, *Spathosternum prasiniferum* walker. *Indian J. Exp. Biol.* 11:351–352.

Salgo, M. P., and G. Oster. 1974. Fetal resorption induced by disulfiram in rats. *J. Reprod. Fert.* 39:375–377.

Saquy, P. C., G. Maia-Campos, M. D. Sousa-Neto, L. F. Guimarães, and J. D. Pécora. 1994. Evaluation of chelating action of EDTA in association with Dakin's solution. *Braz. Dent. J.* 5:65–70.

Sata, F., S. Araki, T. Sakai et al. 1997. Immunological effects of CaEDTA injection: Observations in two lead workers. *Am. J. Ind. Med.* 32:674–680.

Sax, N. I. 1979. *Dangerous properties of industrial materials*, 5th ed., 629, 658. New York, NY: Van Nostrand Reinhold.

Schanker, L. S., and J. M. Johnson. 1961. Increased intestinal absorption of foreign organic compounds in the presence of ethylenediaminetetraacetic acid (EDTA). *Biochem. Pharmacol*. 8:421–422.

Schardein, J. L., R. Sakowski, J. Petrere, and R. R. Humphrey. 1981. Teratogenesis studies with EDTA and its salts in rats. *Toxicol. Appl. Pharmacol*. 61:423–428.

Schoenwald, R. D., and W. L. Ward. 1976. Effect of edetate disodium and reduced glutathione on absorption of acetazolamide from GI tract of rats. *J. Pharm. Sci*. 65:677–680.

Schwartz, S. L., J. R. Hayes, R. S. Ide, C. B. Johnson, and P. D. Doolan. 1966. Studies of ethylenediaminetetraacetic acid. *Biochem. Pharmacol*. 15:377–389.

Schwartz, S. L., C. B. Johnson, J. R. Hayes, and P. D. Doolan. 1967. Subcellular localization of ethylenediaminetetraacetate in the proximal tubular cell of the rat kidney. *Biochem. Pharmacol*. 16:2413–2419.

Segura, J. J., J. R. Calvo, J. M. Guerrero et al. 1996. The disodium salt of EDTA inhibits the binding of vasoactive intestinal peptide to macrophage membranes: Endodontic implications. *J. Endodontics* 22:337–340.

Sinkha, S. P. 1965. Effect of EDTA on crossing over in various lines of Drosophila melanogaster. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 25:T725–T727 (reprinted from *Vestnik Leningradskogo Universiteta, Seriya Biologii* 9(2):130).

Skala, E. 1968. Contribution à l'étude de la toxicité de Na^2 EDTA. *Nouv. Rev. Fr. Hematol*. 8:284–286.

Skala, E., and J. Pospíšil. 1968. Acute toxicity of sodium EDTA in rapid transfusions in dogs. *Physiol. Bohemoslav.* 17:289–295.

Smith, G. 1970. Ethylene diamine tetra-acetic acid and the bactericidal efficiency of some phenolic disinfectants against *Pseudomonas aeruginosa*. *J. Med. Lab. Technol.* 27:203–206.

Spencer, H. 1960. Studies of the effect of chelating agents in man. *Ann. N.Y. Acad. Sci.* 88:435–449.

Sprague, G. 1974–1976. The effect of dietary administration of ethylenediaminetetraacetic acid upon the mineral content of mouse tissues. *Trans. Nebr. Acad. Sci.* 3:61–68.

Srivastava, R. C., P. P. Dwivedi, J. R. Behari, and M. Athar. 1986. Evaluation of LD_{50} of some polyaminocarboxylic acids used as chelating drugs in metal intoxication. *Toxicol. Lett.* 32:37–40.

Sroczyński, J. 1966. The blood-forming system in the course of prolonged prophylactic administration of disodium-calcium versenate in experimental lead poisoning. *Arch. Immunol. Ther. Exp. (Warsz.)* 14:670–677.

Steffensen, D., L. Anderson, and S. Kase. 1956. Crossing over studies with Drosophila and maize. *Genetics* 41:663.

Stevens, E., B. Rosoff, M. Weiner, and H. Spencer. 1962. Metabolism of the chelating agent diethylenetriamine pentaacetic acid (C^{14}DTPA) in man. *Proc. Soc. Exp. Biol. Med.* 111:235–238.

Sugar, A., and S. R. Waltman. 1973. Corneal toxicity of collagenase inhibitors. *Invest. Ophthalmol*. 12:779–782.

Swenerton, H., and L. S. Hurley. 1971. Teratogenic effects of a chelating agent and thier prevention by zinc. *Science* 173:62–64.

Swern, D., ed. 1979. *Bailey's industrial oil and fat products*, Vol. 1, 4th ed., 151–152. New York: John Wiley & Sons.

Takeda, K., F. Katoh, S. Kawai, and K. Konno. 1979. Stimulation of prolyl hydroxylase activity by chelating agents. *Arch. Biochem. Biophys*. 197:273–276.

Taylor, D., and J. Jones. 1972. Effects of ethylenediaminetetraacetate and diethylenetriaminepentaacetate of DNA. Synthesis in kidney and intestinal mucosa of folate treated rats. *Biochem. Pharmacol*. 21:3313–3315.

Taylor, E. J., ed. 1988. *Dorland's illustrated medical dictionary*, 27th ed., 530–531. Philadelphia, PA: WB Saunders.

Teisinger, J., and J. Srbove. 1956. On the question of therapy for chronic lead poisoning with the calcium disodium salt of ethylenediametetraacetic acid. *Arch. Gewerbepathol. Gewerbehyg*. 14:579–593.

Thompson, E. D., B. A. Reeder, R. A. LeBoeuf, M. J. Aardema, D. L. Putman, and M. J. Morris. 1990. Determination of genotoxic and cytotoxic effects of chelators and metal deprivation to CHO cells. *Environ. Mol. Mutagen Suppl.* 15:60.

Tobin, G., A. Aronson, and M. Chvapil. 1974a. Wound healing and collagen degradation in calcium-EDTA-treated rats. *Surg. Forum* 25:49–51.

Tobin, G., A. Aronson, and M. Chvapil. 1974b. Effect of CaEDTA administration on urinary hydroxyproline excretion and skin wound healing in the rat. *J. Surg. Res.* 17:346–351.

Tomita, M., M. Hayashi, and S. Awazu. 1994. Comparison of absorption-enhancing effect between sodium caprate and disodium ethylenediaminetetraacetate in Caco-2 cells. *Biol. Pharm. Bull.* 17:753–775.

Tucker, J. D., and M. L. Christensen. 1987. Effects of anticoagulants upon sister-chromatid exchanges, cell-cycle kinetics, and mitotic index in human peripheral lymphocytes. *Mutat. Res.* 190:225–228.

Umeda, M., K. Noda, and T. Ono. 1980. Inhibition of metabolic cooperation in Chinese hamster cells by various chemicals including tumor promoters. *Jpn. J. Cancer Res.* 71:614–620.

U.S. Pharmacopeial Convention, Inc. 1995. *U.S. Pharmacopeia (USP23) and National Formulary (NF18)*, 570–571, 2012, 2053, 2059, 2243–2244. Rockville, MD: U.S. Pharmacopeial Convention. Inc.

Verplaetse, A., R. M. H. Verbeeck, H. Minnaert, and W. Oosterlinck. 1985. The solubility of inorganic kidneystone components in the presence of acid-base sensitive complexing agents. *Eur. Urol.* 11:44–51.

Verplaetse, H., R. M. H. Verbeeck, A. Verbaeys, and W. Oosterlinck. 1986. Solubility of calcium oxalate monohydrate and hydroxapatite in EDTA solution. *J. Urol.* 135:608–611.

Volf, V., M. Vladar, and A. Seidel. 1971. Distribution of labelled calcium-, yttrium- and chromium-chelates of EDTA in rats. *Arch. Int. Pharmacodyn*. 190:110–123.

Wangenheim, H., and G. Bolcsfoldi. 1988. Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis* 3:193–205.

Weiser, R., A. W. Asscher, and J. Wimpenny. 1968. *In vitro* reversal of antibiotic resistance by ethylenediamine tetraacetic acid. *Nature* 219:1365–1366.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1997. *International cosmetic ingredient dictionary and handbook*, 7th ed., Vol. 1 and 2. 179, 402–403, 467, 496, 600, 1387, 1398–1399, 1436–1438. Washington, DC: CTFA.

Wickramaratne, G. A. de S. 1987. The Chernoff-Kavlock assay: Its validation and application in rats. *Teratog. Carcinog. Mutagen* 7:73–83.

Wilk, A. I., C. T. G. King, and R. M. Pratt, Jr. 1978. Enhancement of chlorcyclizine teratogenicity in the rat by coadministration of calcium chelating agents. *Teratology* 18:193–198.

Wilson, L. A. 1970. Chelation in experimental Pseudomonas keratitis. *Br. J. Opthal.* 54:587–593.

Winder, P. R., and A. C. Curtis. 1960. Edathamil in the treatment of scleroderma and calcinosis cutis. *Arch. Derm.* 82:732–736.

Windsor, E., and G. E. Cronheim. 1961. Gastro-intestinal absorption of heparin and synthetic heparinoids. *Nature* 190:263.

Wynn, J. E., B. Van't Riet, and J. F. Borzelleca. 1970. The toxicity and pharmacodynamics of EGTA: Oral administration to rats and comparisons with EDTA. *Toxicol. Appl. Pharmacol.* 16:807–817.

Yakuji Nippo, Ltd. 1979. *Japanese standards of cosmetic ingredients*, 102–103. Tokyo, Japan: Yakuji Nippo, Ltd.

Yamaguchi, H., H. Kaku, and M. Morisada. 1993a. Experimental pulmonary hypertension induced by constriction of the pulmonary arteries resulting in rupture or extraordinary dilatation of the right ventricle after administration of a small amount of Na_2EDTA. *Exp. Toxicol. Pathol.* 45:21–27.

Yamaguchi, H., H. Kaku, and M. Morisada. 1993b. Study of morphological manifestations of pulmonary arteries in experimental pulmonary hypertension provoked by administration of long-term, low-dose Na_2EDTA-especially in aneurysmal changes and plexiform-like lesions. *Exp. Toxicol. Pathol.* 45:329–335.

Yamaguchi, H., H. Kaku, T. Onodera, R. Kurokawa, and M. Morisada. 1994a. Experimental idiopahtic dilated cardiomyopathy under low-calcium condition. *Exp. Toxicol. Pathol.* 46:223–227.

Yamaguchi, H., M. Morisada, H. Kaku, T. Onodera, and R. Kurokawa. 1994b. Necklace-like detachment of endothelial cell layer from arterial wall under low-calcium condidion. *Exp. Toxicol. Pathol.* 46:307–313.

Yamamoto, A., T. Uchiyama, R. Nishikawa, T. Fujita, and S. Muranishi. 1996. Effectiveness and toxicity screening of various absorption enhancers in the rat small intestine: Effects of absorption enhancers on the intestinal absorption of phenol red and the release of protein and phospholipids from the intestinal membrane. *J. Pharm. Pharmacol.* 48:1285–1289.

Yamashita, S., H. Saitoh, K. Nakanishi et al. 1987. Effects of diclofenac sodium and disodium ethylenediaminetetraacetat e on electrical parameters of the mucosal membrane and their relation to the permeability enhancing effects in the rat jejunum. *J. Pharm. Pharmacol.* 39:621–626.

Yang, S.-S., and M. S. Chan. 1964. Summaries of toxicological data: Toxicity of EDTA. *Food Cosmet. Toxicol.* 2:763–767.

Yonezawa, M. 1977. Basic studies of the intestinal absorption. I. Changes in the rabbit intestinal mucosa after exposure to various surfactants. *Nihon Univ. J. Med.* 19:125–141.

Zeiger E., B. Anderson, S. Haworth, T. Lawlor, and K. Mortelmans. 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagenesis* 11(Suppl 12):1–158.