woodstream

69 N. Locust Street
P.O. Box 327
Lititz, PA 17543-0327
(717)626-2125
Fax: (717)626-1912
www.woodstreamcorp.com
www.saferbrand.com
www.victorpest.com
www.havahart.com
www.perkypet.com
www.fishock.com

RECEIVED
USDA NATIONAL
ORGANIC PROGRAM

2006 APR 13 P 3:04

April, 12, 2006

Robert Pooler
USDA/AMS/TM/NOP
1400 Independence Avenue S.W.
Room 4008 South
Washington, DC 20250

Re: Submission of Petition for Inclusion of Sodium Ferric Hydroxy EDTA on the NOSB List Under the Category: Synthetic substance's allowed for use in organic crop production.

Dear Mr. Pooler:

Enclosed is Woodstream Corporations submission of the petition for the inclusion of the technical ingredient, sodium ferric hydroxy EDTA, on the NOSB List under the category: "Synthetic substance's allowed for use in organic crop production. We are petition the use of this material as an acceptable synthetic substance for use as a molluscicide with no restriction.

This application includes 2 copies of our petition. One copy is a CBI Copy that discloses confidential business information (CBI) in Section 5, as well as in Appendixes B, C, and D of the petition. The front page of the CBI Copy has been labeled "CBI Copy". Each page containing confidential information has been labeled in red "CBI Copy" in the upper right hand corner. All CBI within the text is outlined with brackets and labeled in the right margin with "CBI". This text is not suitable for public distribution or anyone else who does not require access to it. The information in the CBI sections discloses trade secrets and as such is highly confidential. This information was released by Woodstream under the condition that all who view it hold it under strict confidence.

The second copy is a "CBI-deleted" copy and has been clearly labeled on the front page as such. Each page in the CBI sections has been labeled in red with "CBI-deleted". All CBI within the text is deleted. In the right margin, the place where the CBI material has been deleted has been marked with a bracket and labeled in red with "CBI-deleted". In some instances, several pages have been CBI-deleted. In these situations, a single page is used stating that the information has been CBI-deleted and lists the numbers of the deleted pages where the information would have appeared. This copy contains non-confidential information and is available for inspection by members of the public.

If you have any questions or need additional information, please contact Charles Levey via the contact information located on page 1 of the petition.

Sincerely,

David L. Anderson
Product Development Manager
Woodstream Corporation

ORGANIC PETITION

SODIUM FERRIC HYDROXY EDTA

TECHNICAL ACTIVE INGREDIENT

Petitioner: Woodstream Corporation
69 North Locust Street
Lititz, PA 17543

Contact: Charles Levey
Phone: 717-626-2125 x 425
Fax: 717-626-1918
Email: CLevey@Woodstream.com

Petition for inclusion of sodium ferric hydroxy EDTA on the NOSB list under the category:

(1) Synthetic substance's allowed for use in organic crop production.

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2006 APR 13 P 3:01

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ORGANIC PETITION
SODIUM FERRIC HYDROXY EDTA
TECHNICAL ACTIVE INGREDIENT

1. SUBSTANCE NAME:

Sodium Ferric Hydroxy EDTA – CAS # 24594-29-4: It is comprised of iron in a sodium EDTA chelate.

2. MANUFACTURER'S NAME, ADDRESS AND TELEPHONE NUMBER:

Sodium Ferric Hydroxy EDTA is formed during the pellet manufacturing process where Ferric Sodium EDTA binds with free hydroxy molecules to create the active ingredient.

The manufacturer of the end product is:
Woodstream Corporation
69 North Locust Street
Lititz, PA 17543
717-626-2125

The manufacturer of Ferric Sodium EDTA is:

Akzo-Nobel Functional Chemicals LLC
525 West Van Buren Street
Chicago, IL 60607-3823
800-906-7979

3. INTENDED USE:

Pesticide (Molluscicide, as a slug and snail bait)

4. LIST OF THE CROPS FOR WHICH THE SUBSTANCE WILL BE USED, APPLICATION METHOD AND APPLICATION RATE:

Vegetables including (but not limited to): asparagus, artichokes, beans, beets, blackeyed peas, broccoli, Brussels sprouts, cabbage, cantaloupe, carrots, cauliflower, corn, cucumbers, eggplants, garlic, lettuce, onions, peas, peppers, potatoes, radishes, rutabagas, spinach, squash, Swiss chard, tomatoes, turnips.

Orchard Fruits including (but not limited to): apples, avocados, apricots, cherries, grapes, melons, peaches, plums, nectarines, citrus, and pears.

Berries including (but not limited to): strawberries, blackberries, blueberries, boysenberries, loganberries, and raspberries.

Field Crops including (but not limited to): beans, field corn, sweet corn, soybeans, sugarbeets, sugar cane, asparagus, artichokes, beets, broccoli, Brussels sprouts, cabbage, carrots, cauliflower, cucumbers, lettuce, onions, peas, peppers, potatoes, radishes, strawberries, tomatoes, and turnips.

Vineyards

Greenhouse grown vegetables

Grass Grown for Seed Production, Wheat

Indoor Container- Greenhouses

Outdoor Container-Grown Nursery Plants

Turf, golf courses, sod farms

The end-use product, Slug & Snail Killer can be applied by hand or by using broadcast or granular spreaders.

The rate of application for commercial agriculture uses in vegetables, orchards, berries, field crops, vineyards, greenhouses grown vegetables, grass grown for seed production, indoor containers, outdoor containers, and turf is 20 – 40 lbs per acre. For indoor containers ½ teaspoon of bait can be applied in or around a 9-inch pot. For outdoor containers, 1 teaspoon per container is recommended. For smaller areas of turf and ornamentals, 1 lb per 2000 square feet is recommended.

In the Home & Garden Market, the end use product, Snail & Slug bait is applied at a rate of 1 lb per 2000 square feet, or 1 teaspoon per square yard. The bait can be used on lawns, flowers, shrubs, trees, vegetables and fruits.

5. SOURCES:

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6. SUMMARY OF PREVIOUS REVIEWS

No submission has been presented to any organic certification programs.

7. REGISTRATION INFORMATION

EPA Registration

Sodium Ferric Hydroxy EDTA is not currently registered. EPA registration was applied for on December 17th, 2004. Approval is anticipated in the summer of 2006.

Food and Drug Administration Registration

The U.S. Food and Drug Administration designated ferric sodium EDTA, the precursor to sodium ferric hydroxy EDTA, as Generally Recognized as Safe per GRAS notice number GRN 000152.

State Regulatory Authority Registration

Sodium ferric hydroxy EDTA has not been registered by any states. State registrations will be submitted once EPA registration has been approved.

8. CHEMICAL ABSTRACT SERVICE (CAS) NUMBER AND LABELS

CAS# 24594-29-4

Labels

The master label for the end-use Slug & Snail Bait can be found in Appendix A. The User Recommendation Sheet and product details for ferric sodium EDTA can be found in Appendix B.

9. PHYSCICAL PROPERTIES AND MODE OF ACTION

Physical Properties

Sodium ferric hydroxy EDTA is comprised of iron in a sodium EDTA chelate. It is formed during the pellet manufacturing process by the reaction of ferric sodium

EDTA and hydroxy molecules. The technical grade ferric sodium EDTA material is a water-soluble, light greenish yellow powder, with no odor. It contains 13% iron. Ferric sodium EDTA is a commodity ingredient used in the photographic industry as a bleaching agent, used i
n agriculture as a fertilizer micronutrient, and used in the chemical industry as a catalyst. In addition, it is also used as food supplement to prevent anemia and iron deficiencies in developing countries.

Mode of Action

The mode of action is not completely clear but is believed that the active substance is one of the iron ions. The interchange of iron between oxidation states II and III in biological systems is rapid and occurs easily making it difficult to isolate which ion, Fe^{2+} or Fe^{3+}, is responsible for activity.

Most mollusk blood contains a respiratory pigment called hemocyanin, a copper compound. When oxygenated, such blood is bluish in color; when deoxygenated the blood is colorless. The toxic effect of iron (II) or iron (III) ions is due to the fact that they are absorbed on the hemocyanin of the oxygen carrier of the mollusk and this absorption changes the pH reducing the oxygen carrying capacity of the blood leading to suffocation.

a) Chemical interaction with other substances

Sodium ferric hydroxy EDTA is a stable material that is unlikely to react with any other products used in organic farming. Iron is a micronutrient required for plant growth. Sodium ferric hydroxy EDTA is a form of iron that is plant available. Any sodium ferric hydroxy EDTA that is solubilized, is rapidly taken up by plants and therefore, not available to react with other organic inputs. In alkaline soils, the iron is likely to be replaced by an alkaline earth metal such as Ca^{2+} or Mg^{2+}. As iron is ubiquitous in the environment it will react with substances in the soil to form compounds that commonly occur in the soil. No detrimental chemical interactions with other materials used in organic farming are expected to occur.

b) Toxicity and Environmental Persistence

Sodium ferric hydroxy EDTA does not pose a hazard to the environment. This conclusion is based on the facts that: a) the active metabolites, Fe^{2+} and Fe^{3+} are essential for plant and animal growth, b) Iron is applied as a trace element to soil in large tonnages on a worldwide basis, quite often as EDTA, c) Ferric sodium EDTA is used as a dietary supplement and is considered GRAS by the USDA, d) EPA classifies ferric sodium EDTA "as not a biochemical, but eligible for a reduced data set", e) ferric sodium EDTA is on EPA list 4B inert ingredient, f) The application rate adds a fraction of the iron present in the soil, g) Sodium Ferric Hydroxy EDTA is unlikely to accumulate in the environment.

Iron is an essential nutrient for plant and animal growth. It plays a key role as a component of enzymes involved in redox reactions, chlorophyll synthesis, DNA synthesis, oxygen transport, and many other cellular functions. Iron deficiencies in plants lead to interveinal chlorosis, premature leaf fall, dieback of new growth, and stunting. In mammals, iron deficiency symptoms include pallor, weakness, and shortness of breath.

"Plants obtain iron from the soil primarily in the ferric form (Fe^{3+}) as oxides such as $Fe(OH)_2^+$, Fe(OH), and $Fe(OH)_4^-$. This form of iron is extremely insoluble. It is therefore difficult for plant roots to obtain iron from the soil" (Taiz/Zeiger, 1991). As a result, tons of iron is applied to the soil as a trace element fertilizer supplement. "When iron is applied as an inorganic salt such as FeSo4 or Fe(NO3)2, it can form insoluble precipitates. This precipitation of iron makes it unavailable for the plant" (Taiz/Zeiger, 1991). To avoid this problem, iron has been combined with chelating agents like EDTA. "Iron-EDTA complexes are commonly used in nutrient solutions for plants grown hydroponically, aeroponically, or in the soil" (Taiz/Zeiger, 1991).

The FDA has approved the components of sodium ferric hydroxy EDTA as direct food additives. Iron is an essential element for nutrition and is listed as GRAS for direct addition to food per 21 CFR 184.1375. Sodium EDTA is a common chelating agent, which immobilizes metal ions until in an environment where they are available for uptake. Sodium EDTA is a direct food additive per 21 CFR 172.135. A published safety assessment on ferric sodium EDTA for FDA GRAS evaluation concluded that it is regarded as safe when used as a source of dietary iron for food fortification purposes (Tarantino, 2004). In addition, the World Health Organization (WHO) evaluated ferric sodium EDTA and concluded that it was considered safe for use in food fortification programs. Dietary exposure from sodium ferric hydroxy EDTA, as proposed is minimal. Sodium ferric hydroxy EDTA is intended for application to soil surfaces in agricultural crops, turf and ornamentals, and home gardens. The end use product is not applied directly to fruits, vegetables, or plant surfaces.

Sodium ferric hydroxy EDTA has been submitted for EPA registration and is currently under review. EPA has classified ferric sodium EDTA as "Not a biochemical, but eligible for a reduced data set" per the Agency's letter received May 16, 2001. EPA states the classification is based on the abundance of iron in nature, its low toxicity, its use as a nutritional supplement, and its low water solubility.

Ferric sodium EDTA has been identified as an EPA List 4B inert ingredient. In making the List 4B determination, EPA evaluated the toxicity of the chemical substance and considered the exposure that could occur from its use. Based on this evaluation, EPA concluded that the current use of ferric sodium EDTA in pesticides would not adversely affect public health and the environment. In

addition, sodium EDTA, when used in pesticide formulations, is exempt from registration per 40 CFR 180.1001.

Iron is the fourth most abundant element and the second most abundant metal in the Earth's crystal rocks. The normal range of iron concentration in the soil is between 5000 to 50,000 pm (0.5 to 5%) (Brady 1974). Depending upon whether the soil has been cultivated or not, a typical A horizon soil bulk density can range from 0.91 to 1.36 g/cc (Thompson & Troeh 1973). Based on a soil depth of 18cm, $1m^2$ of typical soil weighs between 163,800g and 244,800g and contains between 819 to 12,240g Fe. Following the highest application rate of the end use product (40lbs/Acre), Slug and Snail bait provides $0.035g/m^2$ of iron.

The amount of iron applied to soils as fertilizers is far greater then the amount of iron added with an application of Slug and Snail bait. An application of Slug and Snail bait adds 0.035g/m2 to the soil. The recommended rate for using iron chelates to correct iron deficiencies in field crops is between 0.56 to 1.12g Fe/m2. Following the recommended rates to correct iron deficiency, one application of iron fertilizer applies 16 to 32 times more iron when compared to the highest application rate of Slug and Snail bait.

Sodium ferric hydroxy EDTA is unlikely to accumulate in the environment. As previously mentioned, sodium ferric hydroxy EDTA is rapidly absorbed and utilized by plants. The purpose of Slug and Snail bait is to control land based mollusks that are attacking plants. As a result, plants will take up the majority of the product applied to a soil. The sodium ferric hydroxy EDTA that is not absorbed by plants will breakdown in the environment.

Sodium ferric hydroxy EDTA will react relatively easily to release the ferric ion and take up another metal ion. The ease of this reaction depends strongly upon the pH. In fact, one of the major problems with the agricultural use of ferric EDTA as a source of iron is the ease with which such reactions occur in alkaline soils. The iron usually is replaced by an alkaline earth metal such as Ca^{2+} or Mg^{2+}. Once the iron ion is displaced, it will soon react to form ferrous hydroxide or other very insoluble compounds like those already in the soil. As Iron is ubiquitous in the environment, the ferric ions present in Slug and Snail bait will not pose a hazard.

In considering the fate of the EDTA complex in the soil it is necessary to consider the breakdown due to radiation, bacterial action, and chemical reactions. The majority of EDTA will be degraded photochemically or absorbed by plants as the complex or as Fe3+ chelated by some of the natural root exudates in the rhizosphere and a lesser portion will be degraded in the soil by bacteria. A very small part may find its way into the surface water.

The most important breakdown of ferric EDTA occurs at the air/water interface, which is when the pellet is physically breaking down as a result of water

penetration. Sail and slug pellets are applied to the soil surface under very moist conditions. Over a period of time, typically between 1 to 4 weeks the pellet physically breaks down exposing the inside of the pellet to sunlight. Under these conditions the rate of degradation will approach that of photochemical degradation in a very thin layer of water.

Based on the work of Frank and Rau (1990) and Svenson et al. (1989) the half-life of Fe(III) EDTA in two German rivers has been estimated to be between 2 – 24 hours and between 10 – 480 hours at depths of 70 cm to 2 m. There is obviously a large variation in the rates of photodegradation but the belief is that in aqueous media at or near the surface, such as will exist when the pellet is physically disintegrating, under a range of climatic conditions, the half-life of sodium ferric hydroxy EDTA at the surface of the pellet will be between 20 minutes to 24 hours. Based on a half-life of 12 hours, the concentration drops to 1% after 4 days and to less than 10^{-5} after 1 week.

Sodium ferric hydroxy EDTA that leaches out of the pellet will further degrade in the soil. In the soil, EDTA may exist primarily as the Fe(III) chelate in acidic soils and as the Ca chelate in alkaline soils (HSDB 2004). One of the important aspects of the end-use product is that it contains an appreciable amount of calcium. This has the effect of reacting to replace ferric EDTA with Calcium EDTA and hydrated iron oxide. These reactions will occur in the soil, in water, and in the pellet at the soil/air interface under moist conditions.

Biodegradation is the predominant removal mechanism for EDTA in aerobic soils. The usual route of degradation is by successive loss of –COOH groups from the EDTA moiety. Studies by Tiedje (1975a, 1975b) indicate that accumulation of FeEDTA in the environment over years is unlikely. This worker found that when FeEDTA is present at relatively low concentrations (2 to 1000 g/g in the soil) it is biologically removed from soil and sediments. They found that low concentrations of EDTA and its metal chelates disappeared over periods ranging from 15 to 45 weeks, in a wide variety of soils. Although no single bacteria capable of metabolizing EDTA or of metal chelates was isolated, it was postulated that co-metabolism by a mixed population was the most probable mechanism.

Belly and co-workers (1975) found that mixed cultures of aerobic bacteria were capable of degrading over 90% of ferric EDTA in 5 days, for cultures containing less than 2 millimolar EDTA. These researchers found that a mixed population of microorganisms present in an aerobic lagoon biologically degraded ferric EDTA. They found that 28% of acetate-2-C and 30% of the ethylene carbon was recovered as carbon dioxide after 5 days. Intermediaries for ferric EDTA degradation are: ethylenediamine triacetic acid (ED3A), iminodiacetic acid (IDA), ethylenediamine diacetic acid (N,N-EDDA), ethylenediamine monoacetic acid (EDMA), nitilotriacetic acid (NTA), and glycine.

If released into water, EDTA may react with photochemically-generated hydroxyl radicals (half-life, 229 days) or undergo photodegradation. In aqueous solution, the Fe(III) complex of EDTA has been reported to undergo photodegradation with a half-life of 11.3 minutes (HSDB 2004). EDTA is not expected to adsorb to suspended solids and sediment (HSDB 2004). The high water solubility, rapid microbial degradation, and low to moderate bioconcentration factor indicate that EDTA would not be expected to bioaccumulate in aquatic organisms (HSDB 2004).

c) Environmental Impacts from Use or Manufacture

The environment would not be impacted as a result of the use of sodium ferric hydroxy EDTA in the Slug and Snail bait end-use product (see section 9b).

As described in section 5, sodium EDTA is manufactured by reacting ethylenediamine, hydrogen cyanide, formaldehyde, sulfuric acid, hydrogen peroxide, sodium hydroxide and water to form tetra sodium EDTA.

The tetra sodium salt is then reacted with ferric sulfate to give ferric sodium EDTA. The solid product is produced by crystallization.

The only by-products of the manufacturing process are ammonia and sodium sulfate. Both by-products occur naturally in nature.

During the pellet manufacturing process, ferric sodium EDTA reacts with free hydroxy molecules to form sodium ferric hydroxy EDTA.

d) Effects on Human Health

Sodium ferric hydroxy EDTA is of low risk to human health. The precursor, ferric sodium EDTA, is a commodity ingredient used as a food supplement to prevent anemia and iron deficiencies, used in agriculture as a micronutrient, and used in the photographic industry as a bleaching agent.

Iron is essential to the metabolism of plants and animals. Iron is involved in oxygen transport, electron transfer, DNA synthesis and many other cellular functions. As humans cannot easily metabolize iron from food sources, and iron deficiency is a common disease, iron supplementation of food often occurs.

Ferric sodium EDTA has been shown to have significant beneficial effects on iron status by increasing iron bioavailability in human diets (Hembach, 2000). In 2000, a World Health Organization (WHO) committee reviewing the use of ferric sodium EDTA as a food supplement concluded it to be safe for use in food

fortification programs. Iron is listed as GRAS for direct addition to food per 21 CFR 184.1375. Sodium EDTA is a direct food additive per 21 CFR 172.135. A published safety assessment on ferric sodium EDTA for FDA GRAS evaluation concluded that it is regarded as safe when used as a source of dietary iron for food fortification purposes (Tarantino, 2004). The recommend daily allowance of iron for adults is 10 – 18 mg. WHO has set the daily human intake of iron from ferric sodium EDTA to be 0.2mg/kg body weight in food fortification programs (WHO 2000). Ferric sodium EDTA is significantly more expensive than other sources of iron and as a result, it is not widely used in fortification programs (Hurrell, 1997).

"Iron EDTA, like other EDTA-metal complexes, dissociates in the gastrointestinal tract to form iron, which is bioavailable, and an EDTA salt; absorption of the metal ion and EDTA are independent. Because of this dissociation, consideration of information on EDTA compounds other than iron EDTA is relevant."(Hembach, 2000).

"EDTA compounds are poorly absorbed in the gastrointestinal tract and do not undergo significant metabolic conversion" (Hembach, 2000). In fact, 95% of the EDTA molecule is excreted in the stool (Hurrell, 1997). As a result, EDTA compounds have a low degree of oral toxicity. Toxicological studies have been conducted to evaluate the toxicity of ferric sodium EDTA. In chronic toxicity studies, diets containing as much as 5% EDTA were without adverse effects. Acute oral tests found no adverse effects were seen in rats that received an oral gavage dose of 5,000 mg/kg body weight (mg/kg bwt) of ferric sodium EDTA. No rats died during the 14 day observation period, and no gross pathological changes were found in organs in the thoracic or abdominal cavities at necropsy. A LD50 >5,000 mg/kg was established (see appendix C).

EDTA compounds have a very low dermal absorption rate. Only 0.001% of CaNa2EDTA is absorbed after dermal application (Institute for Health and Consumer Protection, 2004). Acute dermal toxicity tests with ferric sodium EDTA resulted in no adverse effects in rats that received a dermal dose of 5,000 mg/kg bwt. No effects on appearance, behavior or body weight were observed in any rats any time after exposure. No rats died during the 14-day observation period, and no gross pathological changes were found in organs in the thoracic or abdominal cavities at necropsy. A LD50 >5,000 mg/kg was established (see appendix C).

Inhalation of EDTA compounds is not expected to be toxic (Institute for Health and Consumer Protection, 2004). Acute inhalation toxicity trials with rats resulted in no adverse effects when exposed by inhalation for 4 hours to a concentration of 2.05 mg/L of ferric sodium EDTA. No effects on appearance, behavior, or body weight were observed in any rats any time after exposure. No rats died during the 14-day observation period, and no gross pathological changes were found in organs in the thoracic or abdominal cavities at necropsy. A LD50>2.05 mg/L was established (see appendix C).

EDTA compounds are considered to be irritating to the eyes (Institute for Health and Consumer Protection, 2004). The cause of the irritation is due to the formation of an acid pH when the compound is dissolved in water. In a primary eye irritation study on rabbits, ferric sodium EDTA was classified as mildly irritating to the eye (see appendix C).

EDTA compounds are not carcinogenic in experimental animal bioassays and are not directly genotoxic (Hembach, 2000). The potential for ferric sodium EDTA to cause an allergic response is expected to be extremely low. In primary skin irritation study on rabbits, ferric sodium EDTA was classified as slightly irritating to the skin (see appendix C). In addition, a dermal sensitization study on guinea pigs found that ferric sodium EDTA was not considered to be a contact sensitizer (see appendix C).

Chronic iron overload in humans has been known to occur. "Acute iron toxicity has typically resulted from accidental ingestion of medicinal iron or adult iron supplements by children. Symptoms of iron toxicity include lethargy, nausea and vomiting, abdominal pain, black stools, and signs of shock, as well as metabolic acidosis, liver damage and coagulation defects that may occur several days after ingestion. Delayed effects include renal failure and hepatic cirrhosis." Toxicity caused by long-term use of iron is more common in adults than children, but is generally limited to individuals with metabolic disorders affecting maintenance of iron balance (Heimbach, 2000). Poisoning symptoms may occur from Iron overload caused by the acute ingestion of as little as 25 mg/kg body weight/day, with clinically significant iron poisoning occurring at iron doses of 60 mg/kg bw/d (Tarantino, 2004). To achieve a 60 mg/kg bw/d, it is estimated that a 10 kg child (22 lbs) would need to consume approximately 77 grams of the end-use Slug and Snail bait. This estimate supports the conclusion that sodium ferric hydroxy EDTA will not cause iron overload.

Toxicological studies show that ferric sodium EDTA does not pose a threat to human health. The FDA considers it generally recognized as safe and the World Health Organization has reviewed it and allowed its use as an iron supplement. Therefore, it can be concluded that adverse health effects would be unlikely to occur from exposure to sodium ferric hydroxy EDTA and even less likely from and end-use Slug and Snail bait containing 5.87% sodium ferric hydroxy EDTA.

e) Effects on Soil Organisms, Crops or Livestock

Sodium ferric hydroxy EDTA is not expected to cause adverse effects on soil organisms, crops or livestock. This conclusion is based on a) iron being ubiquitous in the environment, b) the known role iron plays in plants and animals, c) the use of ferric sodium EDTA as a fertilizer, d) the use of ferric sodium EDTA as a dietary supplement, e) the use pattern of the end-use product, f) the lack of propensity for ferric sodium EDTA to bioaccumulate, and g) the toxicological data on sodium ferric hydroxy EDTA.

Iron is the fourth most abundant element and the second most abundant metal in the Earth's crystal rocks. As iron is so common in the environment, the incremental amount added as a result of an application of Slug and Snail bait is not expected to have adverse effects on avian, mammalian, or aquatic populations. Any free iron from sodium ferric hydroxy EDTA will quickly react with substances in the soil to form compounds that commonly occur in the soil.

Iron is an essential nutrient for plant and animal growth. In plants, it plays a key role as a component of enzymes involved in redox reactions, chlorophyll synthesis and many other cellular functions. In mammals, iron functions in oxidative enzyme systems involved in energy metabolism. It also enables the hemoglobin in red blood cells to carry oxygen. Iron deficiencies in plants lead to interveinal chlorosis, premature leaf fall, dieback of new growth, and stunting. In mammals, iron deficiency symptoms include pallor, weakness, and shortness of breath.

Iron is commonly applied to crops as a fertilizer supplement. Iron deficiency is difficult to correct because of rapid transformation of iron contained in fertilizers to unavailable forms in the soil. Ferric sodium EDTA is a chelated form of iron that is used to keep the iron available for plant uptake. The recommended rate for using iron chelates to correct iron deficiencies in field crops is between 0.56 to 1.12g Fe/m2. An application of Slug and Snail bait adds 0.035g/m2 to the soil. The additional amount of iron provided from Slug and Snail bait is a fraction of the amount applied as a fertilizer supplement.

Anemia due to iron deficiency is a common problem throughout the world. To combat this, supplemental iron is added to foods or taken as tablets. In 2000, a World Health Organization (WHO) committee reviewing the use of ferric sodium EDTA as a food supplement concluded it to be safe for use in food fortification programs. Iron is listed as GRAS for direct addition to food per 21 CFR 184.1375. Sodium EDTA is a direct food additive per 21 CFR 172.135. A published safety assessment on ferric sodium EDTA for FDA GRAS evaluation concluded that it is regarded as safe when used as a source of dietary iron for food fortification purposes (Tarantino, 2004).

As mentioned previously, Ferric sodium EDTA has been identified as an EPA List 4B inert ingredient. In addition, all of the other ingredients present in the end-use product are on either EPA list 4A or 4B. List 4A and 4B inert ingredients are not expected to adversely affect the environment. List 4A ingredients are considered to have very low toxicity or are practically non-toxic and are considered safe for use in all pesticide products. EPA has concluded that List 4B ingredients would not adversely affect public health and the environment as currently used.

Sodium ferric hydroxy EDTA does not bioaccumulate. In order to bioaccumulate a substance has to be more soluble in cell material than in aqueous media and the solubility in aqueous media has to be low. (i.e. less than 10 mg/L). Sodium ferric hydroxy EDTA is quite soluble in water as are the degradation products. Therefore, it cannot bioaccumulate in fish or other animals. It appears that any metabolite of the EDTA moiety will contain at least one –COOH group and be water-soluble. Bioaccumulation of the Fe moiety is not feasible.

Ferric sodium EDTA is used as an ingredient in several aquarium products indicating that it is not toxic to the aquatic organisms. Non-target toxicity tests have shown that ferric sodium EDTA does not appear to pose a significant hazard. Of the species tested, only the water flea was less than 100 ppm. Based on the non-target toxicity testing conducted on a diverse population of organisms, adverse effects are not expected from use of sodium ferric hydroxy EDTA. See summaries below:

STUDY	RESULTS
Avian Acute Oral- Bobwhite quail	LD50>2038 mg/kg[a]
Non-target – Water flea	LC50 32mg/L[b]
Non-target - Flatworm	LC50>100 mg/L[b]
Non-target – Snail	LC50>100 mg/L[b]
Non-target – Minnow	LC50>100mg/L[b]
Non-target – Sideswimmer	LC50>100mg/L[b]
Non-target – Pillbug	LC50>100mg/L[b]
Non-target – Segmented worm	LC50>100mg/L[b]

a. See appendix D
b. Ewell, 1996

Non-target toxicity trials were conducted on the end-use Slug and Snail bait (Young, unpublished) and are summarized below:

STUDY	RESULTS
Non-target – Ladybird larvae	No significant effects – non toxic
Non-target – Carabid beetle	No significant effects – non toxic
Non-target – Melyrid beetle	No significant effects – non toxic

10. SAFETY INFORMATION

The material safety data sheet for ferric sodium EDTA is found in appendix B.

A National Institute of Environmental Health Studies substance report on sodium ferric hydroxy EDTA is not available.

11. RESEARCH INFORMATION, INCLUDING RESEARCH REVIEWS AND BIBLIOGRAPHIES

a) Belly, R.T., Lauff, J.J., Goodhue, C.T., 1975. Degradation of Ethylenediaminetetraacetic acid by Microbial Populations from an Aerated Lagoon. Appl. Microbio. 29, 787-794.

b) Boucher, J. Slugs. General pest management article. Integrated Pest Management. University of Connecticut.

c) Brady, N. C. 1974. The Nature and Properties of Soils. 8th Edition. Macmillan Publishing Co., Inc., New York.

d) Jones, R. S. U.S. Environmental Protection Agency. 2001. EPA Agency Response Letter. U.S. EPA.

e) Ewell, W.S., Gorsuch, J.W., Kringle, R.O., Robillard, K.A., Speigel, R.C., 1986. Simultaneous Evaluation of the Acute Effects of Chemicals on Seven Aquatic Species. Environ. Toxicology and Chemistry. 5, 831-840.

f) Frank, R., Rau, H. 1990. Photochemical Transformation in Aqueous Solution and Possible Environmental Fate of Ethylenediaminetetraacetic Acid (EDTA). Ecotoxicology and Environmental Safety 19:55-63.

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12. PETITION JUSTIFICATION STATEMENT

Sodium ferric hydroxy EDTA is consistent with organic farming and handling practices and its use should be allowed in organic production. The justifications for inclusion on the national list are: a) slugs and snails cause serious damage to crops and control methods are needed to prevent economic losses; b) alternative controls are available however they are unreliable and inefficient; c) Sodium ferric hydroxy EDTA effectively kills slugs and snails, yet rather than being harmful to humans, animals, and plants, it actually provides benefits; d) a similar synthetic molluscicide, ferric phosphate, was recently included on the list; and e) ferric sodium EDTA is an EPA List 4 inert ingredient and as such would be allowed in organic production as a pesticide inert ingredient.

Slugs and snails can cause significant damage to plants resulting in serious farm, home garden and greenhouse losses. These mollusks damage plants by eating the leaves and tender shoots or leaving a slime trail that detracts from the appearance of the produce. Slugs and snails will feed on almost all crops including grains, clovers, corn, young vegetables, ornamentals and small fruits. They are especially attracted to ripe strawberries and tomatoes (Nielson, 2003). The economic threshold level for slugs and snails numbers is crop dependant. Depending on the crop being grown, a catch of 1 to 5 per monitoring trap indicates a risk of economically significant damage and that control is needed (Boucher).

Cultural methods are used to control slugs and snails but they have their limitations. Barriers: Copper tape and diatomaceous earth are used to create a barrier that slugs and snails will not cross. The problem with barriers is that environmental factors can disrupt their effect. Diatomaceous earth is no longer effect once it is wet and must be reapplied. Barriers will lose their effectiveness if vegetation or debris bridges the barrier or if a gap exists that the slug or snail can crawl under. In addition, great care must be taken to be sure that the mollusks are not on the inside of the area being protected.

Biological Controls: (insects and birds) Carabid beetles will feed on slugs and snails and are capable of reducing pest populations. Unfortunately, these controls also have their weaknesses. Carabid beetles are unlikely to be useful in protecting field vegetables as pupae are often killed during cultivation and mollusks will use the plant as a refuge from attack (Glen, 2002). Many bird species will eat slugs and snails. However, they cannot provide short-term control and they are also capable of damaging crops (Glen, 2002).

Physical Controls: (ie. beer traps and hand picking). Beer traps sunk into the soil will effectively capture slugs. However, there are problems. For this method to be effective, traps must be spaced every meter and the traps must be maintained every few days (Symondson, 2001). Hand picking works well for eliminating large slugs, but not the small ones. This process can be improved by using slug shelters and by collecting at night. Both of these options require a great deal of labor and are impractical for large areas.

Cultural controls (i.e. cultivation and removal of potential shelters) Cultivating the soil will kill slugs and expose them and their eggs to predators and removal of crop residues will eliminate slug harborages reducing slug populations. Timing of these practices is critical to be effective. Organic growers utilizing no-tillage practices or using mulches will find these controls impractical.

Caffeine: Caffeine has been found to kill and repel slugs. However, studies have shown that it is toxic to some earthworm species and it is phytotoxic to plants.

Repellents: (Aluminum Sulfate and Copper Sulfate) aluminum sulfate and copper sulfate are only effective against very small slugs. They are quickly washed down with rain, so repeated applications are often necessary. Caution must be exercised with their use as copper sulfate is toxic to fish and water plants and can be detrimental to earthworms. In addition, aluminum sulfate has been linked to human health problems and it is toxic to plants.

Baits: The only molluscicide bait available to organic growers is ferric phosphate. This material was just recently added to the National List as a synthetic substance allowed for use in organic crop production with no restrictions. This material is similar to sodium ferric hydroxy EDTA. According to the patent, the end use product also contains a synthetic chelating agent (U.S. Patent #5,437,870). The toxicity profiles are comparable and they use the same mode of action to control mollusks. The problem with ferric phosphate as a molluscicide is that there is only one basic manufacturer of the bait and as such a monopoly has been created for the organic market. An alternative is needed to create competition and keep the costs affordable to growers.

Sodium ferric hydroxy EDTA is an effective control method that has a low toxicity level to other organisms. This safety is based on the fact that the mode of action is highly specific to mollusks and that ferric sodium EDTA is recognized to be safe. To be effective, it must first be consumed by the organism. The bait is used in a manner and is formulated such that mollusks are the most likely organism to consume it. Sodium ferric hydroxy EDTA is highly specific to these organisms. It works by disrupting the oxygen carrying capacity of the mollusks copper based blood. Humans and most other organisms utilize a different circulatory process and as a result do not experience the toxic effects. The USDA considers ferric sodium EDTA to be generally recognized as safe and they, along with the World Health Organization, allow its use as a dietary supplement.

Toxicity tests have shown that sodium ferric hydroxy EDTA will not harm non-target organisms. The EPA has determined that ferric sodium EDTA would not adversely affect public health and the environment. Iron is one of the most common minerals in the environment yet at times it is limiting in plant nutrition. The iron in sodium ferric hydroxy EDTA is in a form that is highly available to plants and ferric sodium EDTA is used as a fertilizer supplement.

On March 2, 2005 the National Organic Standards Board set a precedent when it recommended to the National Organic Program that ferric phosphate be added to section 205.601 of the National List. The rationale for the decision was "The material is consistent with OFPA and meets the three criteria – minimal impact on humans and environment, no non-synthetic substance is available for mollusk control, and the material is compatible and consistent with organic production practices." Sodium ferric hydroxy EDTA is similar to ferric phosphate; they are synthetic, they have the same mode of action, they have similar use patterns, they contain synthetic chelates, and they have low levels of toxicity to non-target organisms.

Both ferric phosphate and sodium ferric hydroxy EDTA molluscicides are man made. The manufacturing processes are similar in that the both have sodium sulfate and water as by-products. The only difference is that the production of sodium ferric hydroxy EDTA also results in ammonia as a by-product. The ferric phosphate and sodium ferric hydroxy EDTA are combined with other inert ingredients and formed into a bait pellet.

The mode of action for ferric phosphate and sodium ferric hydroxy EDTA is identical. In both cases, an iron compound is ingested and absorbed by the mollusk. The iron ions move into the blood stream and then bind to hemocyanin. Hemocyanin is a copper compound that functions as the oxygen carrier. When iron binds to the hemocyanin, the pH is changed and the oxygen carrying capacity is reduced. The ultimate effect is that the mollusk dies from suffocation.

The ferric phosphate and sodium ferric hydroxy EDTA baits also have similar use patterns. Product is applied either by broadcasting material over a large area or used as a spot treatment for small areas. The main difference is in application rate. The ferric phosphate based bait is applied at a rate of 1 lb/1000 ft2 while sodium ferric hydroxy EDTA bait is applied at a rate of 0.5 lb/1000 ft2.

Sodium ferric hydroxy EDTA and iron phosphate baits contain a synthetic chelating agent. Although the exact formula used in the ferric phosphate product is not in the public domain, the patent is (US Patent #5,437,870), and it discloses that the bait must contain a synthetic chelating agent to be effective. The patent specifically identifies edetic acid, hydroxyethyl derivative of edetic acid, or salts therof.

The toxicity of sodium ferric hydroxy EDTA and ferric phosphate are comparable. Companies registering pesticides with EPA are required to conduct specific toxicology studies. The studies include acute oral toxicity, acute dermal toxicity, dermal sensitization, ocular irritation, dermal sensitivity, and acute inhalation. LD50s are

determined for the acute toxicity trials and ratings are generated for the others. EPA takes these data and places them into categories based on set criteria. Tests conducted on ferric sodium EDTA and ferric phosphate resulted in the products receiving the exact same EPA category ratings. In addition, ferric phosphate and sodium ferric hydroxy EDTA were used in non-target toxicity trials conducted on Bobwhite quail. Both materials had LD50s greater then 2000mg/kg.

The final justification for adding sodium ferric hydroxy EDTA to the National List is that its precursor, ferric sodium EDTA, is on EPA List 4 of inert ingredients. Section 205.601 M (1) EPA List 4 - Inerts of Minimal Concern allows the use of synthetic inert ingredients in pesticides for use with non-synthetic substances or synthetic substances listed in section 205.601 and used as an active pesticide ingredient in accordance with any limitations on the use of such substances. Ferric sodium EDTA is included on EPA List 4 and therefore if it were used as inert ingredient instead of a precursor for the active ingredient, it would be allowed for use in organic production. It is possible that ferric sodium EDTA is currently being applied to organic crops as an inert pesticide ingredient.

Based on the facts that slugs and snails cause serious damage to crops and control methods are needed, that alternative controls are available but are unreliable and inefficient, that sodium ferric hydroxyl EDTA is an effective molluscicide that provides benefits to crops, that ferric phosphate, a similar molluscicide has recently been allowed for organic crop use, and that the precursor to sodium ferric hydroxy EDTA is allowed for organic crop use as an inert ingredient, we request that sodium ferric hydroxy EDTA be added to the National List as a synthetic substance allowed for use in organic crop production as a molluscicide with no restriction.

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Degradation of Ethylenediaminetetraacetic Acid by Microbial Populations from an Aerated Lagoon

R. T. BELLY,* J. J. LAUFF, AND C. T. GOODHUE

Research Laboratories, Eastman Kodak Company, Rochester, New York 14650

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The ferric chelate of ethylenediaminetetraacetic acid (EDTA) was biologically degraded by a mixed population of microorganisms present in an aerated lagoon receiving this chemical in its feed. As determined radiorespirometrically, 28% of the acetate-2-C and 30% of the ethylene position of the ammonium ferric chelate of [^{14}C]EDTA was recovered as $^{14}CO_2$ after 5 days. In a separate experiment using gas liquid chromatography and the sodium ferric chelate, as much as 89% disappearance of EDTA (0.1% wt/vol) was observed during a similar time period. Optimum $^{14}CO_2$ evolution was observed at a pH value between 7 and 8 and at room temperature. Degradation of NH_4Fe-[2-^{14}C]EDTA was stimulated by the addition of either unlabeled NaFe-EDTA, nitrilotriacetic acid or ethylenediamine, and inhibited by the addition of a variety of different sugars and amino acids. Consistent with the biological nature of this degradation, little or no $^{14}CO_2$ evolution was observed after heat treatment of the microorganisms at 100 C for 10 min, or after the addition of formalin or antibiotics to the incubation mixtures. Gas-liquid chromatography and mass spectral analyses were performed to demonstrate EDTA disappearance and to identify various possible intermediates of EDTA degradation.

Ethylenediaminetetraacetic acid (EDTA) and its metal chelates are used widely in a variety of agricultural and industrial processes. The consensus expressed in earlier literature indicated that EDTA was recalcitrant to biological degradation (1, 7, 11). However, more recent studies by Tiedje and his associates (J. M. Tiedje, E. S. Perry, and T. S. Savage, Abstr. Annu. Meet. Am. Soc. Microbiol., p. 5, 1974), indicate that EDTA is degraded slowly by the microorganisms present in a variety of different agricultural soils. These workers reported that both the ethylene and acetate parts of the EDTA molecule are attacked in this degradation, and that the degradation of EDTA is strongly inhibited by microbial inhibitors.

In addition to the possible biological degradation of EDTA, several reports have indicated that the ferric chelate of EDTA is photodegraded at wavelengths between 250 and 400 nm (13, 14). Further studies by Natarajan and Endicott (16) have identified the products of this photolysis as CO_2, formaldehyde, and ferrous iron.

Because of the similarity in structure between nitrilotriacetic acid (NTA) and EDTA, insight into the mechanism of degradation of EDTA can be obtained from a review of the literature concerning the biochemistry of NTA metabolism. Such studies have shown that NTA is degraded to CO_2 and biomass with the release of the N atom as NH_4^+ (12). Sequential induction studies have suggested that the metabolism of NTA proceeds by the successive removal of two carbon fragments to form iminodiacetic acid (IDA) and glycine (20), and not by a mechanism involving decarboxylation and the formation of *N*-methyliminodiacetate or *N*-methylglycines. Recent evidence of Cripps and Noble (9) indicates that the oxidation of NTA to IDA is mediated by a nicotinamide adenine dinucleotide, reduced form, and O_2-dependent enzyme, and that growth on NTA results in increased activities of the enzymes of glycine and serine metabolism. The end products of NTA degradation reported by these workers are 2 mol of glyoxylate and 1 mol of glycine per mol of NTA degraded. Both glyoxylate and glycine are metabolized to glycerate.

In the present study, we investigated the microbial degradation of the sodium- or ammonium-ferric chelate of EDTA (Na- or NH_4Fe-EDTA) by mixed populations of microorganisms present in an aerated lagoon receiving this chemical. Degradation of acetate- or ethylene-labeled NH_4Fe-EDTA was determined

initially by a radiorespirometric technique, and confirmed by total organic carbon (TOC) and gas-liquid chromatographic (GLC) methods. Possible intermediates in the degradation of EDTA were identified by GLC and mass spectral analyses.

MATERIALS AND METHODS

Biological materials. Water samples from an aerated lagoon receiving EDTA-containing industrial effluents, located in Webster, N.Y., were collected in 5-gallon (ca. 19 liters) containers and stored overnight at 3 C. In early experiments, biological material was harvested with a table-top centrifuge at 7,000 × *g* for 10 min. When large quantities of cellular material were required, a refrigerated continuous centrifuge (CEPA, type 41-G) operated at 19,000 rpm was used. After centrifugation, the clear supernatant fluid was discarded, and the cell pellet was suspended in Allen basal salt medium (2) at pH 7.

Chemicals. Ethylenediaminemonoacetic acid (EDMA) was synthesized according to the procedure of Atherton et al. (3), and *N,N*-ethylenediaminediacetic acid (*N,N*-EDDA) according to the procedure of Schwarzenbach et al. (18). The cobalt chelate of ethylenediaminetriacetic acid (ED3A) was synthesized by the technique of Blackmer et al. (5). For mass spectral studies, nonchelated ED3A was obtained from C. R. Flynn of the Research Laboratories, Eastman Kodak Co. All other chemicals were of the highest purity available from commercial sources.

NH_4Fe-[2-^{14}C]EDTA (0.86 mCi/mg) and NH_4Fe-EDTA-[^{14}C]ethylene (0.60 mCi/mg) were synthesized from either EDTA-[2-^{14}C]acetate or EDTA-[^{14}C]-ethylene (Mallinckrodt Chemical Works, St. Louis) according to the procedure of Brintzinger et al. (6). The resultant chemicals were recrystallized in acetone-water (4:1). To test the purity of the radiochemicals, a portion of each chemical was spotted on a silica-gel chromatographic sheet without fluorescent indicator (Eastman Organic Chemicals, no. 6061) and developed with a solvent of either ethanol, NH_4OH, acetic acid (7:3:1), or ethanol, NH_4OH (2:1). Autoradiography was performed on the resultant chromatogram by means of Kodak single-coated medical X-ray film—blue sensitive. The purity of both radiochemicals was estimated to be better than 95% according to this procedure.

Radiorespirometric incubations. Either 1 or 2 ml of biological material was placed in a 30-ml capacity serum bottle containing 2 ml of salts solution (2) and 0.2 ml of 1 M potassium phosphate buffer, pH 7.3. The serum bottles were sealed with rubber stoppers, and either 0.1 or 0.2 ml of radioactive substrate was injected through the stopper. To prevent leakage of gas from the vial, the vials were coated with 731RTV adhesive (Dow Corning Co.). Samples were incubated routinely in the dark at room temperature.

The reactions were stopped by the addition of 2 ml of 1 N perchloric acid to each serum vial. In addition to killing bacterial cells, the perchloric acid acidifies the reaction, releasing $^{14}CO_2$ generated into the gaseous phase.

To trap $^{14}CO_2$ produced in the reaction, both a long cannula (7.6-cm 20-gauge needle) and a short cannula (3.8-cm 16-gauge needle) were placed through the rubber stopper of the vial. Attached to the longer cannula was an air source; attached to the shorter cannula were two scintillation vials containing 12 ml of scintillation fluid and phenethylamine (19). Air was bubbled through the vials at a flow rate of about 2 bubbles/s. After 7 min, the first vial was removed from the apparatus and counted. Virtually all $^{14}CO_2$ was recovered in this first vial. The second vial served as a safety trap for $^{14}CO_2$.

Radioactive samples were counted by using a Packard Tricarb 3375 liquid scintillation spectrometer. In early studies, Aquasol Universal LSC Cocktail (New England Nuclear Corp., Boston, Mass.) was used for counting aqueous samples. However, in later studies Eastman ready-to-use II (Eastman Kodak Co., Rochester, N.Y.) scintillation fluid was used.

Identification of intermediates in degradation. For the identification of possible intermediates in the degradation of EDTA, 300 ml of Allen basal salts medium (2) containing 0.1% (wt/vol) NaFe-EDTA was placed in each of three 2.8-liter Fernbach flasks. The flasks were autoclaved for 15 min at 121 C. Flask 1 was not inoculated and served as an uninoculated control. Flask 2 contained cell suspension, and 30 ml of added formaldehyde solution. This flask served as a formaldehyde control. Flask 3 served as an experimental and contained 3.59 mg/ml (dry wt) (final concentration) of cell suspension. To preclude the possibility of photodegradation, the flasks were incubated in the dark at room temperature.

Five- or ten-ml samples were removed from the flasks and centrifuged at 7,000 × *g* for 10 min. The supernatant fluid was kept frozen until analysis.

Total nonvolatile organic carbon analysis. Total nonvolatile carbon analyses were performed on 1-ml samples acidified with 25 μl of 3 N HCl with an Envirotech TOC analyzer, model no. DC-50. The limit of detection for these analyses was 1 ppm of nonvolatile carbon with an accuracy and a precision of 5%.

GLC and mass spectral analyses. Samples for GLC analysis were acidified with HCl and stored frozen until the time of analysis. EDTA and ED3A analyses were performed by drying samples under nitrogen and then causing the residue to react with boron fluoride (Eastman Organic Chemicals, no. 3706) to form the methyl esters according to the procedure of Rudling (17). One-microliter portions were chromatographed on a Barber-Coleman 5000 gas chromatograph equipped with either a 4-ft or a 6-ft (121.92 or 188.88 cm) glass U-tube packed with 3% (wt/wt) diethyleneglycol succinate on Chromosorb W. Solutions containing known concentrations of either EDTA (disodium salt) or Fe-EDTA (monosodium salt) and ED3A (cobalt chelate) were prepared, methylated, and chromatographed as standards. With flame ionization detection, the limits of detection for EDTA and ED3A were less than 0.1 mM.

Glycine, IDA, NTA, EDMA, *N,N*-EDDA, and *N,N'*-EDDA were analyzed as their *n*-butyltrifluoroacetyl derivatives formed by reaction of samples

evaporated to dryness under nit
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were dried and then caused to re
trifluoroacetic anhydride in Cl
flame ionization detection each
could be detected at less than
(wt/wt) diethyleneglycol adipat
addition experiments confirmed
NTA, EDMA, glycine, *N,N*-EDI
in experimental samples.

The *n*-butyltrifluoroacetyl d
and ED3A formed in experimen
used for their quantitative analy
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adipate column. Furthermore,
ED3A (cobalt chelate) did not
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Mass spectra were obtained
double-focusing mass spectrom
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formed from standard samples
EDMA, NTA, IDA, *N,N*-EDDA
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RESULTS

Initial studies demonstrat
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covery was observed after
Therefore, both the ethylene
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degraded in this process.

Effect of pH and tempera
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evaporated to dryness under nitrogen with 3 N hydrochloric acid-butanol at 85 C. The butylated samples were dried and then caused to react with 25% (vol/vol) trifluoroacetic anhydride in CH_2Cl_2 (4, 21). With flame ionization detection each of these compounds could be detected at less than 0.01 mM on a 5% (wt/wt) diethyleneglycol adipate column. Standard addition experiments confirmed the presence of IDA, NTA, EDMA, glycine, *N,N*-EDDA, and *N,N'*-EDDA in experimental samples.

The *n*-butyltrifluoroacetyl derivatives of EDTA and ED3A formed in experimental samples were not used for their quantitative analysis since these derivatives eluted very late from the 5% diethyleneglycol adipate column. Furthermore, standard samples of ED3A (cobalt chelate) did not react quantitatively under the mild butylation conditions used.

Mass spectra were obtained on a CEC-21-110B double-focusing mass spectrometer with Mattauch geometry. *N*-butyl-trifluoroacetyl derivatives were formed from standard samples of EDTA, ED3A, EDMA, NTA, IDA, *N,N*-EDDA, *N,N'*-EDDA, and glycine. In some cases impurities and reagent residues obscured their lower mass fragments. A derivatized portion from the 4-day biodegradation sample was introduced into the inlet of the mass spectrometer and several scans were taken as the inlet temperature was gradually increased. This procedure provided a partial separation of components of different volatility. Parent peaks as well as at least two other unique fragments were observed for each compound except glycine. However, peaks characteristic of glycine but not unique to this compound were observed in the biodegradation sample.

RESULTS

Initial studies demonstrating degradation of acetate- and ethylene-labeled EDTA. Degradation of NH_4Fe-EDTA was observed initially in a series of experiments in which either acetate- or ethylene-labeled NH_4Fe-[^{14}C]EDTA was added to reaction mixtures containing buffer, a salts solution, and biological material from the aerated lagoon. After various incubation periods in the dark, the extent of $^{14}CO_2$ formation in each sample was determined radiorespirometrically. As shown in Fig. 1, 27% of the initial radioactivity of the acetate-labeled NH_4Fe[^{14}C]EDTA was recovered as $^{14}CO_2$ after a 5-day incubation period; and in the case of ethylene-labeled compound, a 31% $^{14}CO_2$ recovery was observed after this time period. Therefore, both the ethylene backbone and the acetate side chains of the EDTA molecules are degraded in this process.

Effect of pH and temperature on NH_4Fe-[^{14}C]EDTA degradation. The following buffers were used to determine the effect of pH on degradation: 1,4-piperazinediethanesulfonic acid at pH values between 5 and 7; *N*-2-hydroxyethylpiperazine - *N'* - 2 - ethanesulfonic acid at pH 7.5; *N*-tris(hydroxymethyl)methylglycine at pH 8.0; and tris(hydroxymethyl)-methylaminopropanesulfonic acid at pH 8.5 and 9.0. Buffers were added at a final concentration of 0.2 M and the vials incubated for 5 days at room temperature. Degradation occurred at all pH values tested between 5 and 9; optimum was between 7 and 8. Of the three temperatures tested at pH 7.3, the best for degradation was room temperature (ca. 22 C). There was a 98% decrease in $^{14}CO_2$ evolution at 3 C, and a 49% decrease in $^{14}CO_2$ evolution at 37 C.

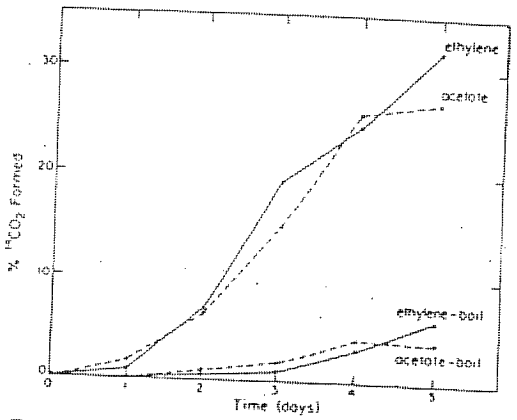

FIG. 1. $^{14}CO_2$ *evolution from acetate- and ethylene-labeled* NH_4Fe[^{14}C]*EDTA. Each 30-ml serum bottle contained 0.2 ml of 1 M potassium phosphate buffer, pH 7.3, 1 ml of Allen basal salts, 1 ml of cell suspension, and either 0.2 ml of* NH_4Fe-[2-^{14}C]*EDTA (acetate) (78,500 counts/min) or 0.2 ml of* NH_4Fe-*EDTA-*[^{14}C]*ethylene (94,600 counts/min). All vials were incubated in the dark at room temperature, and each point represents an average of triplicate determinations. Controls were heated at 100 C for 10 min before the addition of the radioactivity.*

Effect of various carbon sources on NH_4Fe-EDTA degradation. It seemed possible that auxiliary carbon sources might stimulate NH_4Fe-EDTA degradation. Various carbon sources were added at a final concentration of 0.1% to a reaction mixture containing biological material from the aerated lagoon and NH_4Fe-[2-^{14}C]EDTA. Incubation conditions are as described in Fig. 1. These experiments indicated after 5 days a 2.7-fold increase of $^{14}CO_2$ evolution in the presence of added NaFe-EDTA over control vials containing no additions. Increases also were observed when either NTA (1.8-fold) or ethylenediamine (1.2-fold) was added to the standard reaction mixtures. In contrast to the stimulation observed with the above compounds, the addition of several amino acids (e.g., L-glutamate, L-aspartate, L-serine, L-alanine, glycine) and sugars (e.g., D-fructose, D-

glucose, and glycerol) strongly inhibited degradation.

Effect of various microbial inhibitors on degradation. To obtain additional evidence indicating that EDTA degradation is biological, and to gain some insight into the types of microorganisms involved, a study of the effect of various antibiotics and other inhibitors on the degradation was undertaken. A strong inhibition of $^{14}CO_2$ evolution was observed after a 5-day incubation with a variety of antibiotics including novobiocin (98% at 1 mg/ml), chloramphenicol (86% at 11,100 U/ml), streptomycin (82% at 2,470 U/ml), cycloheximide (71% at 1 mg/ml), penicillin G (59% at 5,560 U/ml), and formaldehyde (100% at 8% vol/vol). Samples that were heated at 100 C for 10 min before the addition of the radioactive substrate demonstrated an 83% inhibition of $^{14}CO_2$ evolution over untreated controls.

EDTA disappearance by TOC and GLC analyses. Since previous evidence for EDTA degradation was based on results obtained by means of a radiorespirometric technique, we verified degradation of this compound by other techniques including total organic carbon analysis (TOC) (Fig. 2). NaFe-EDTA (0.1% [wt/vol]) was the sole carbon source present in the flask. Therefore, a decrease in total nonvolatile organic carbon is indicative of EDTA utilization. A reduction of 63% in total nonvolatile organic carbon was observed after 5 days.

EDTA disappearance in the flask also was measured by a GLC method quantitative for this chemical. These experiments, summarized in Fig. 3A, demonstrated a decrease in EDTA concentration from 2.6 mM to 0.29 mM during a 5-day incubation period.

Identification of possible products and intermediates of EDTA degradation. Various compounds in reaction mixtures containing EDTA and aerated lagoon material were identified by a GLC method quantitative for amino polycarboxylic acid (4, 21). These studies are summarized in Fig. 3A and B. Along with a decrease in EDTA concentration, there was a notable increase in the concentrations of both ED3A, and IDA (Fig. 3A). This increase in ED3A and IDA concentrations appeared to reach a maximum at day 2, after which time there was a pronounced decrease in the levels of these possible intermediates. Such decreases presumably can be explained by a further microbial degradation of ED3A and IDA. In control samples, ED3A and IDA were present at less than 0.1 mM.

In contrast to ED3A and IDA, other possible intermediates in the degradation of EDTA were

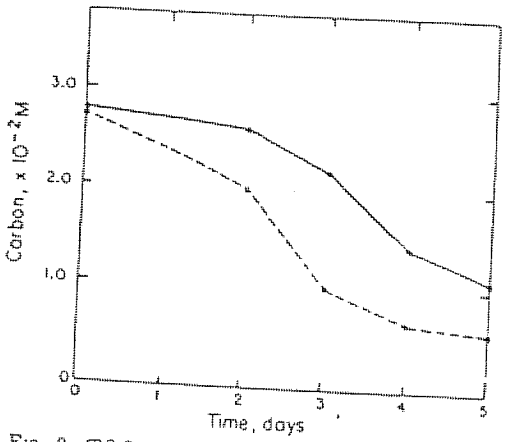

FIG. 2. *TOC and the calculated total of organic carbon present in all identified intermediates. Solid line represents TOC analyses; dotted line represents the sum total of organic carbon for all intermediates detected by GLC (see Fig. 3).*

detected in rather low concentrations (less than 0.065 mM) (Fig. 3B). Included among these compounds were both isomers of ethylenediaminediacetic acid (*N,N*-EDDA, and *N,N'*-EDDA), EDMA, NTA, and glycine. Each of these compounds was detected in 2-day-old samples with maximal concentration occurring at 3 to 5 days. After this time period, a decrease in concentration of these intermediates was observed. In control samples, these chemicals were found at levels less than 0.01 mM.

Mass spectral analyses. To verify the presence of the intermediates demonstrated by GLC analyses, the fragmentation patterns of the butyl ester trifluoroacetyl derivatives were determined as shown in Table 1. The spectra of these known compounds were compared with spectra observed in a similarly derivatized sample from the previous 4-day degradation study (Fig. 3A and B). Molecular ions and unique fragments were obtained for all of the compounds except glycine listed in Table 1.

Calculated versus actual nonvolatile organic carbon. To determine whether all intermediates of EDTA were accounted for in the previous GLC analyses, the total carbon for all intermediates detected by GLC was calculated and compared with total nonvolatile carbon analysis (TOC) of the samples. As shown in Fig. 2, consistently higher amounts of carbon were detected by TOC analysis compared to calculated carbon contents.

DISCUSSION

The present study is the first to demonstrate biological degradation of EDTA in an aquatic

system and to identify pos
this degradation. These
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the EDTA molecule to m
11).

Because of possible und
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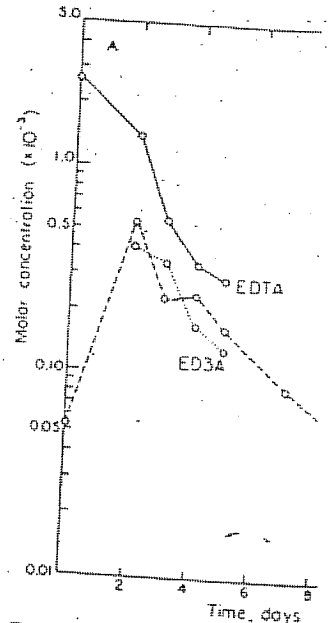
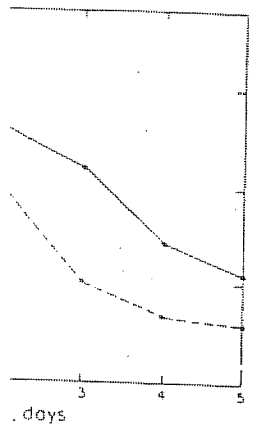


FIG. 3. *(A and B). Possible int
from each flask at the time interv
kept frozen until analysis. Each ex
suspension in addition to a basal
include EDTA, ED3A, glycine, ID*



. days

lculated total of organic ified intermediates. Solid es; dotted line represents rbon for all intermediates 1).

ncentrations (less than included among these isomers of ethylene- ',N-EDDA, and *N,N'-* and glycine. Each detected in 2-day-old oncentration occurring ime period, a decrease se intermediates was ples, these chemicals than 0.01 mM.

es. To verify the pres- demonstrated by GLC tion patterns of the l derivatives were de- ible 1. The spectra of were compared with larly derivatized sam- ay degradation study lar ions and unique for all of the com- ed in Table 1.

tual nonvolatile or- ine whether all inter- accounted for in the e total carbon for all GLC was calculated l nonvolatile carbon ples. As shown in Fig. ounts of carbon were s compared to calcu-

ION

first to demonstrate EDTA in an aquatic

system and to identify possible intermediates in this degradation. These results have significance not only in assessing possible accumulation of this chemical in the environment, but also in changing the consensus expressed in earlier literature indicating the recalcitrance of the EDTA molecule to microbial attack (1, 7, 11).

Because of possible underestimation of degradation with the radiorespirometric technique, both a GLC technique and a total nonvolatile carbon (TOC) analysis were used to measure the extent of breakdown of EDTA. These studies demonstrated approximately 89% degradation of EDTA after 5 days based on the GLC method, and a 63% reduction in TOC during this time. Differences between these two methods probably are attributable to the organic carbon present in various intermediates of degradation as well as to cellular carbon released as a result of lysis during the course of the experiment.

It seems clear that the degradation of NH_4Fe-EDTA reported in the present paper is biological. Several lines of evidence support this conclusion: (i) all incubations were performed in the dark, thereby precluding the possibility of photolysis; (ii) optimum degradation was observed at or near physiological temperatures and pH values; (iii) degradation was stimulated by the addition of possible intermediates of a biological degradation including NTA and ethylenediamine, and inhibited by the addition of various sugars and amino acids; (iv) controls pretreated with heat or with formaldehyde demonstrated little or no activity; (v) degradation was strongly inhibited by several antibiotics known to affect microbial protein synthesis or membrane function.

Bacterial involvement is indicated by the strong inhibition observed with chloramphenicol (86%) and streptomycin (82%). On the other hand, cycloheximide, an antibiotic primarily acting on eucaryotic cells such as algae and fungi demonstrated 71% inhibition. Because adsorption and inactivation of the antibiotics might have occurred during the long duration of our experiments, a rather high concentration of antibiotic was added. Therefore, the extent of involvement of each microbial group is difficult to determine.

Our experiments demonstrating a decrease in $^{14}CO_2$ evolution from NH_4Fe-[^{14}C]EDTA when certain amino acids or sugars are added can be

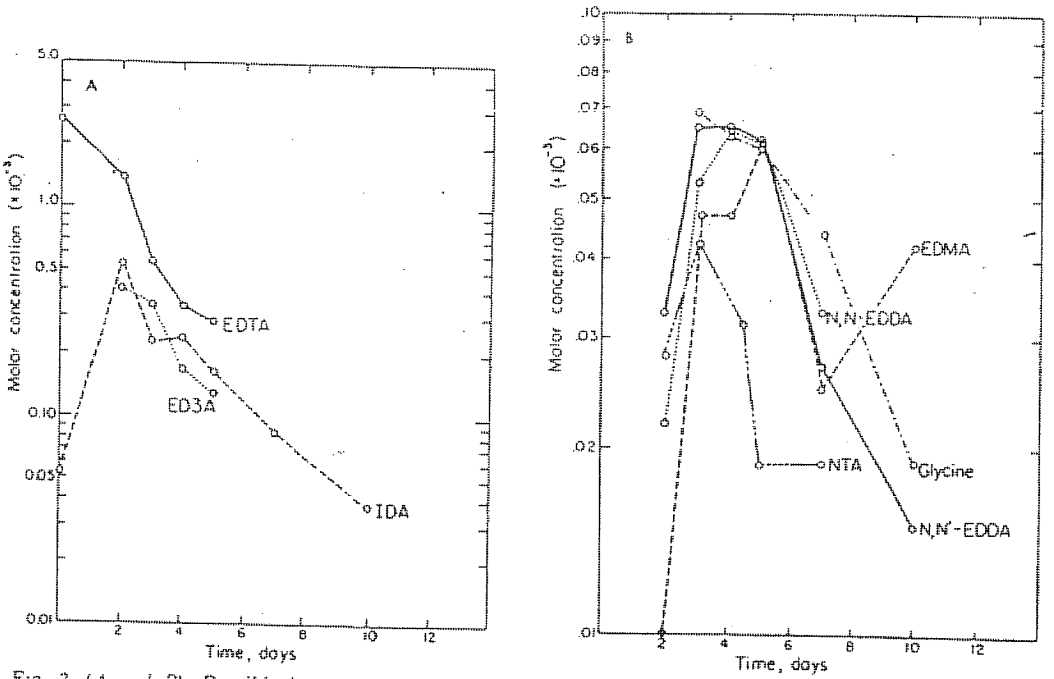
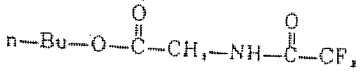
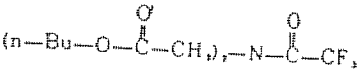
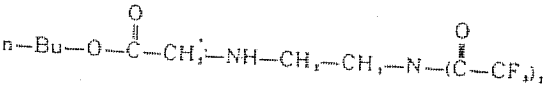
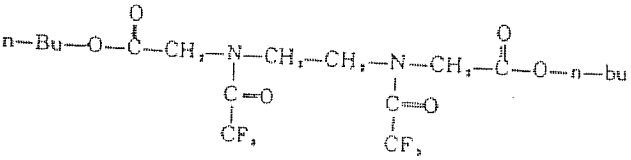
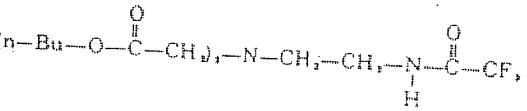
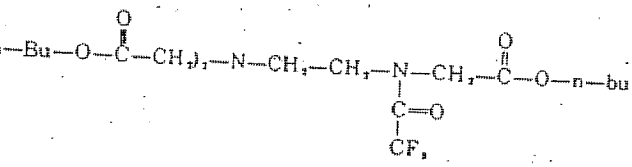
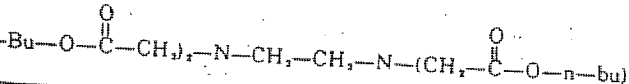


FIG. 3. *(A and B). Possible intermediates of EDTA degradation. Five milliliters of sample was removed from each flask at the time intervals indicated. The samples were centrifuged and the clear supernatant was kept frozen until analysis. Each experimental flask contained 3.59 mg/ml (dry wt) (final concentration) of cell suspension in addition to a basal salts solution and NaFe-EDTA. Intermediates detected by GLC analysis include EDTA, ED3A, glycine, IDA, EDMA, NTA, N-EDDA, and N,N'-EDDA.*

TABLE 1. *Mass spectral analysis for various possible intermediates*[a]

Compound[b]	Structure of derivative	Partial fragmentation pattern (*m/e*)
Glycine		228, 198, 172, 154, 152, 127, 126
IDA		341,[c] 267, 229, 211, 184
EDMA		366,[c] 292, 265, 253, 240, 228, 184, 139, 126
N,N'-EDDA		480,[c] 406, 379, 310, 253, 184, 181
N,N-EDDA		384,[c] 310, 283, 258
NTA		359,[c] 285, 258, 158, 144
ED3A		498[c], 397, 328, 258, 227, 199
EDTA		516[c], 442, 415, 258, 227, 144

[a] The butyl ester trifluoroacetyl derivatives of known compounds were determined on a CEC-21-110B double-focusing mass spectrometer of Mattauch geometry.
[b] See Fig. 3A and B for details.
[c] Parent peak.

interpreted in several ways. First, the presence of such organic compounds or their metabolites may repress the synthesis of one or more of the enzymes required for EDTA degradation. Second, since many of these sugars and amino acids are rapidly biodegraded, a preferential utilization of such compounds over EDTA may have occurred in reaction vials. And finally, the addition of certain sugars and amino acids may have produced population shifts in the vials resulting in a decrease in the EDTA-utilizing population.

GLC methods confirmed by mass spectral analyses were used to identify possible intermediates and end products of EDTA degradation. These analyses indicate that ED3A and IDA are probably intermediates. In addition, low levels of other possible intermediates, including *N,N*-EDDA, *N,N'*-EDDA, EDMA, NTA, and glycine were observed. It is reasonable that all these intermediates have resulted from the microbial degradation of EDTA, but the possibility that some of these compounds may have been formed during derivatization cannot be eliminated.

Based on the presence of all these compounds, at least two different pathways may be

postulated for the degradatic
mixed population of microor
the aerated lagoon. One path
involves metabolism of the F
the successive removal of
Several other secondary an
have been reported to be
similar oxidative cleavage of
form amine and aldehyde pr
In the case of EDTA, the
products would be ED3A and
then would be metabolized by
bond cleavage, ultimately re
enediamine and glyoxylate
Recent results in our laborat
unpublished data) indicate
amine can be metabolized
sumably, NH_4^+.

Another mechanism is requi
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15), at least three different
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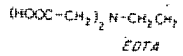

EDTA

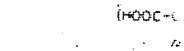
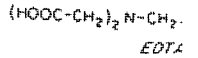
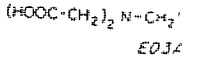


FIG.


EDTA


ED3A

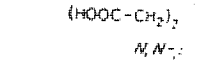


FIG. 5. *Proposed*

diates*

	Partial fragmentation pattern (m/e)
	228, 198, 172, 154, 152, 127, 126
	341,[c] 267, 229, 211, 184
	366,[c] 292, 265, 253, 240, 228, 184, 139, 126
	480,[c] 406, 379, 310, 253, 184, 181
	384,[c] 310, 283, 258
	359,[c] 285, 258, 158, 144
	498[c], 397, 328, 258, 227, 199
	516[c], 442, 415, 258, 227, 144

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n addition, low levels
liates, including *N,N*-
MA, NTA, and glycine
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may have been formed
not be eliminated.
all these compounds,
pathways may be

postulated for the degradation of EDTA by the mixed population of microorganisms present in the aerated lagoon. One pathway shown in Fig. 4 involves metabolism of the EDTA molecule by the successive removal of 2-carbon units. Several other secondary and tertiary amines have been reported to be metabolized by a similar oxidative cleavage of the C—N bond to form amine and aldehyde products (8, 10, 15). In the case of EDTA, the analogous initial products would be ED3A and glyoxylate. ED3A then would be metabolized by successive C—N bond cleavage, ultimately resulting in ethylenediamine and glyoxylate as end products. Recent results in our laboratory (R. T. Belly, unpublished data) indicate that ethylenediamine can be metabolized to CO_2 and presumably, NH_4^+.

Another mechanism is required to explain the occurrence of NTA and IDA in reaction mixtures. Based on reported microbial mechanisms for the cleavage of alkylnitrogen bonds forming an amine and an aldehyde as products (8, 10, 15), at least three different reactions can explain the occurrence of these intermediates (Fig. 5). Whether NTA or its aldehyde is formed biologically from EDTA, ED3A, or *N,N*-EDDA, or all of these chemicals remains to be determined. NTA-aldehyde may be converted to NTA by either a dehydrogenase reaction or by an oxidase reaction. Tiedje et al. (20), and Cripps and Noble (9) have proposed that NTA is metabolized by an oxidative cleavage resulting in the formation of glyoxylate and IDA. IDA then undergoes a similar oxidative cleavage forming glycine and glyoxylate. Both gloxylate and glycine are metabolized readily by a variety of microorganisms to form CO_2; and in the case of glycine, NH_4^+ is released.

Highest concentrations of the possible intermediates of EDTA degradation were obtained between days 2 and 4. After this time, a significant decrease in the levels of many of these compounds was detected. Such a decrease can be explained by metabolism and degradation of the intermediates of EDTA degradation. If this explanation is correct, then the present study indicates that in addition to EDTA, other intermediates such as ED3A, EDDA, and EDMA also can be degraded by microorganisms

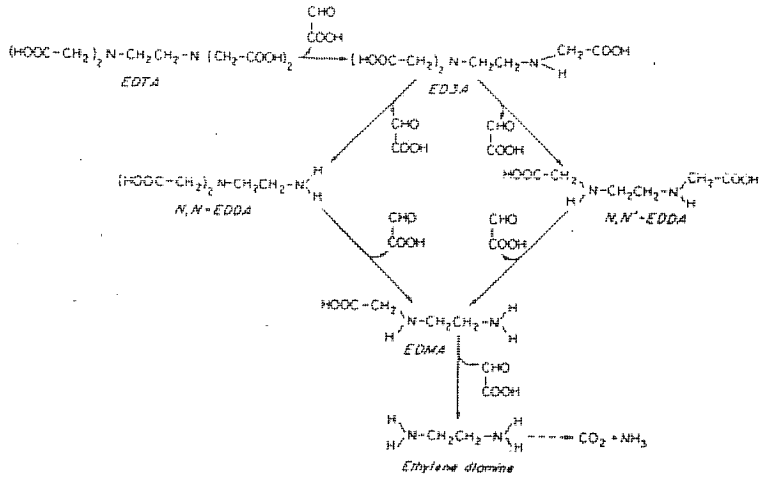

FIG. 4. *A proposed pathway for EDTA degradation.*

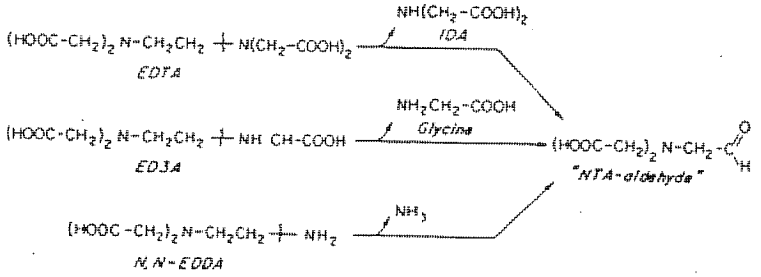

FIG. 5. *Proposed mechanisms for NTA-aldehyde formation from EDTA.*

in aerated lagoons, and is consistent with findings that both the ethylene and acetate parts of the EDTA molecule are metabolized to similar extents.

ACKNOWLEDGMENTS

We thank Elaine Criswell for providing ED3A (cobalt chelate) and EDMA and C. Flynn for providing the ED3A and *N,N*-EDDA. We also thank John Smith for EDTA and ED3A analyses, David Maier for performing mass spectral analyses, and R. T. Ambrose for his helpful advice.

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University of Connecticut
INTEGRATED PEST MANAGEMENT

Slugs

Description and Life History. There are many species of slugs capable of causing economic damage to greenhouse crops and field crops such as crucifers, strawberries, raspberries, potatoes, tomatoes, beans and lettuce. Most species survive the winter as eggs or adults. An adult may be a single sex or have both male and female reproductive organs (hermaphroditic) and be self-fertile. A single individual is capable of producing up to 500 eggs, which are deposited in batches of one to several dozen under stones, debris or in the soil. Eggs usually hatch in the spring or early summer. Slugs are nocturnal feeders, hiding during daylight hours.
During wet growing seasons, large numbers of slugs survive the summer months and may move into cultivated fields from weedy borders, drainage ditches or other moist, sheltered areas. Crop damage is usually most severe in late summer or early autumn of cool, wet growing seasons, especially when preceded by a mild winter. Sizable slug populations may build up in fields previously in no-till corn, alfalfa, potatoes and strawberries.

Damage and Scouting. Slugs feed by grating away the surface of the plant tissue with a tooth-covered radula, which works like a rasp. This type of feeding injury is easily distinguished from caterpillar feeding on thick-leafed cole crops like cabbage. The grating action produces a large wound on the leaf surface nearest the slug, which gradually tapers to a smaller hole through the opposite surface. Slug injury to cabbage appears ragged compared with the clean-sided incision typical of caterpillar feeding. On thin-leafed crucifers or other crops, insects produce leaf injury which is virtually indistinguishable from slug feeding. The presence of a glistening slime trail can sometimes be used to distinguish slug injury. Also, most insect pests can be found on the foliage during daylight hours while slugs tend to hide off the plant. Slugs attack the fruit of tomatoes and strawberries leaving small (1/2-inch long), shallow holes in the fruit's surface.

Scout for signs of slug feeding on crop plants near the weedy borders of fields.

Monitoring. A covered pit can be used to provide a humid, sheltered hiding place for slugs during daylight hours. The pit should be four inches in diameter and six inches deep. An aluminum foil-covered shingle or a board can be used as a cover to provide a cool refuge from the sun. Slugs tend to congregate in large numbers in these shelters and may be counted and destroyed during daylight hours. Set monitoring traps near field borders. The traps will not function as well in weedy fields or with crops like cabbage which provide adequate shelter for slugs beneath large-frame leaves close to the ground.

Action Threshold. The literature suggests applying control measures when one to five slugs per trap are found.

Cultural Control. Maintain good weed control within the field and along borders to eliminate daytime refuges of slugs.

Chemical Control. Baits are much more effective at controlling slugs than are foliar applied insecticides. Therefore, it is important to distinguish between caterpillar and slug injury. Apply baits in a clean cultivated strip along the margins of fields to intercept slugs entering fields from weedy borders.

For crops that provide plenty of foliar cover near ground level (e.g., cabbage), broadcast the bait through the entire field if slug damage is a problem Weeds provide alternative food sources for slugs and will reduce the effectiveness of baits. Metaldehyde baits (e.g., Deadline Bullets) are currently registered for slug control on many crops. Metaldehyde use is prohibited after edible portions of the plant begin to form. Follow label application instructions carefully for best results.

Written by Jude Boucher, Vegetable Crops IPM Program Coordinator, University of Connecticut

menu

THE NATURE AND PROPERTIES OF SOILS *8th Edition*

NYLE C. BRADY

Professor of Soil Science
New York State College of Agriculture and Life Science
Cornell University
and Director, International Rice Research Institute,
Philippines

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Chapter 17

SUPPLY AND AVAILABILITY OF PHOSPHORUS AND POTASSIUM

CONSIDERABLE nitrogen can be added to soils through biochemical fixation brought about by microorganisms. If the proper legume is chosen, for example, the organisms will often fix this element from the air in quantities sufficient to temporarily increase the nitrogen already present. With other nutrient elements, such as phosphorus and potassium, however, there is no such microbial aid. Consequently, other sources must be depended upon to meet the demands of plants.

There are at least four main sources of phosphorus and potassium from which these demands can be met: (a) commercial fertilizer; (b) animal manures; (c) plant residues, including green manures; and (d) native compounds of these elements, both organic and inorganic, already present in the soil. Since the first three sources are to be considered in later chapters, attention now will be focused on the ways and means of utilizing the body of the soil as source of these mineral elements.

17:1. Importance of Phosphorus

With the possible exception of nitrogen, no other element has been as critical in the growth of plants in the field as has phosphorus. A lack of this element is doubly serious since it may prevent other nutrients from being acquired by plants. For example, prior to the extensive usage of commercial fertilizers, most soil nitrogen was indirectly dependent upon the supply of phosphorus. This was due to the vital influence of phosphorus on legume growth. Today the demand for phosphorus by nitrogen-yielding legumes is universally recognized.

The need of plants for phosphorus has been especially considered in the formulation of commercial fertilizers. This element, in the form of superphosphate, was the first to be supplied as a manufactured product. Until fairly recently the amount of "phosphoric acid" in mixed fertilizers almost invariably exceeded that of nitrogen or potash. Even today the total tonnage of phosphorus, expressed as P_2O_5, is exceeded only by that of nitrogen.

17:2. Influence of Phosphorus on Plants

It is difficult to state in detail the functions of phosphorus in the economy of even the simplest plants. Only the more important functions will be considered here. Phosphorus makes its contribution through its favorable effect on the following:

1. Cell division and fat and albumin formation.
2. Flowering and fruiting, including seed formation.
3. Crop maturation, thus counteracting the effects of excess nitrogen applications.
4. Root development, particularly of the lateral and fibrous rootlets.
5. Strength of straw in cereal crops, thus helping to prevent lodging.
6. Crop quality, especially of forages and of vegetables.
7. Resistance to certain diseases.

17:3. The Phosphorus Problem

Although the amount of total phosphorus in an average mineral soil compares favorably with that of nitrogen, it is much lower than potassium, calcium, or magnesium (see Table 2:3). Of even greater importance, however, is the fact that most of the phosphorus present in soils is currently unavailable to plants. Also, when soluble sources of this element are supplied to soils in the form of fertilizers, their phosphorus is often "fixed" or rendered insoluble or unavailable to higher plants, even under the most ideal field conditions (see p. 465).

Fertilizer practices in many areas exemplify the problem of phosphorus availability. As already emphasized, the tonnage of phosphorus-supplying materials used as fertilizers definitely exceeds all except the nitrogen carriers. The removal of phosphorus from soils by crops, however, is low compared to that of nitrogen and potassium, often being only one third or one fourth that of the latter elements. The necessity for high fertilizer dosage when relatively small quantities of phosphorus are being removed from soils indicates that much of the added phosphates becomes unavailable to growing plants.

The influence of this situation on fertilizer practice is clearly shown when considering the additions of fertilizer phosphorus in comparison with crop removal.

In the United States, phosphorus added in fertilizers exceeds that removed by crops by more than 24 percent (see Table 17:1). In some areas, notably the eastern seaboard states, additions of phosphorus more than triple the removal of this element by crops. Since phosphorus is lost only sparingly by leaching, the inefficiency of utilization of phosphate fertilizers is obvious.

Briefly, then, the overall phosphorus problem is threefold: (a) a small total amount present in soils, (b) the unavailability of such native phosphorus, and (c) a marked "fixation" of added soluble phosphates. Since crop removal

of phosphorus is relatively low and world phosphate supplies are huge, problem (a), that of supplying sufficient total phosphorus, is not serious. Increasing the availability of native soil phosphorus and the retardation of fixation or reversion of added phosphates are, therefore, the problems of greatest importance. These two phases will be discussed following a brief review of the phosphorus compounds present in soils.

TABLE 17:1. *Nutrients Removed by Crops in the United States Compared to That Added in Fertilizers (1965)*[a]

	N	P	K
Removed in crops (thousands of tons)	8,838	1,207	4,152
Added in fertilizers (thousands of tons)	4,580	1,499	2,313
Addition as percent of removal	52	124	56

[a] Nutrient removal figures calculated from White (25). Fertilizer additions from Tennessee Valley Authority (23).

17:4. PHOSPHORUS COMPOUNDS IN SOILS[1]

Both inorganic and organic forms of phosphorus occur in soils and both are important to plants as sources of this element. There is a serious lack of information, however, on the relative amounts of these two forms in different soils. Data available from Oregon, Iowa, and Arizona (Table 17:2) give some idea of their relative proportions. Despite the variation which occurs, it is evident that a consideration of soil phosphorus would not be complete unless some attention were given to both forms (see Fig. 17:1).

INORGANIC COMPOUNDS. Most inorganic phosphorus compounds in soils fall into one of two groups: (a) those containing *calcium*, and (b) those containing *iron* and *aluminum*. The calcium compounds of most importance are listed in Table 17:3. Fluorapatite, the most insoluble and unavailable of the group, usually is an original mineral. It is found in even the more weathered soils, especially in their lower horizons. This fact is an indication of the extreme insolubility and consequent unavailability of the phosphorus contained therein. The simpler compounds of calcium, such as mono and dicalcium phosphate, are readily available for plant growth. Except on recently fertilized soils, however, these compounds are present in extremely small quantities only since they easily revert to the more insoluble forms.

Much less is known of the exact constitution of the iron and aluminum phosphates contained in soils. The compounds involved are probably hydroxy phosphates such as dufrenite, wavellite, strengite, and variscite (10). These compounds are most stable in acid soils and are extremely insoluble.

[1] For a review of soil phosphorus, see Larsen (17).

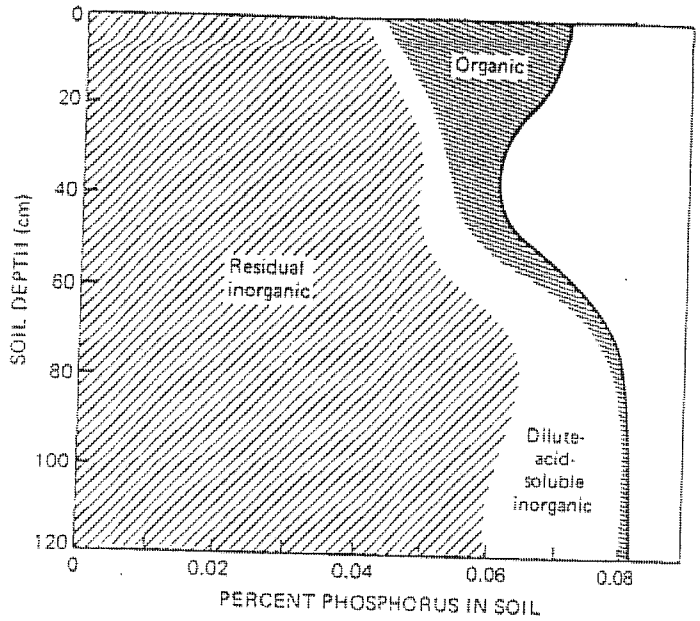


FIGURE 17:1. Distribution of phosphorus in organic and inorganic forms in an Iowa soil. The dilute-acid-soluble inorganic phosphorus is more readily available than the residual inorganic forms. In heavily fertilized soils the upper horizons would likely be much higher in inorganic phosphorus. [*From Black* (6).]

TABLE 17:2. *Total Phosphorus Content of Soils from Three States and the Percentage of Total Phosphorus in the Organic Form*

Soils	Number of Samples	Total P (ppm)	Organic Fraction (%)
Western Oregon soils			
Hill soils	4	357	65.9
Old valley-filling soils	4	1,479	29.4
Recent valley soils	3	848	25.6
Iowa soils			
Prairie soils	2	613	41.6
Gray-brown podzolic soils	2	574	37.3
Planosols	2	495	52.7
Arizona soils			
Surface soils	19	703	36.0
Subsoils	5	125	34.0

[a] Figures for Oregon from Bertramson and Stephenson (5), for Iowa from Pearson and Simonson (19), and for Arizona from Fuller and McGeorge (13).

TABLE 17:3. *Inorganic Calcium Compounds of Phosphorus Often Found in Soils*

Compound	Formula	Solubility increases
Fluor apatite	$3\,Ca_3(PO_4)_2 \cdot CaF_2$	
Carbonate apatite	$3\,Ca_3(PO_4)_2 \cdot CaCO_3$	
Hydroxy apatite	$3\,Ca_3(PO_4)_2 \cdot Ca(OH)_2$	
Oxy apatite	$3\,Ca_3(PO_4)_2 \cdot CaO$	
Tricalcium phosphate	$Ca_3(PO_4)_2$	
Dicalcium phosphate	$CaHPO_4$	
Monocalcium phosphate	$Ca(H_2PO_4)_2$	

Many investigators have shown that phosphates react with certain iron or aluminum silicate minerals such as kaolinite. There is some uncertainty, however, as to the exact form in which this phosphorus is held in the soil. Most evidence indicates that it, too, is probably fixed as iron or aluminum phosphates, such as those described in the preceding paragraph.

ORGANIC PHOSPHORUS COMPOUNDS. There has been relatively less work done on the organic phosphorus compounds in soils, even though this fraction in some cases comprises more than one half of the total soil phosphorus. One of the reasons for lack of information on these compounds is because they apparently are exceedingly complex. The meager data available, however, indicate that the three main groups of organic phosphorus compounds[2] found in plants are also present in soils (7). These are (a) phytin and phytin derivatives, (b) nucleic acids, and (c) phospholipids. There are likely other organic phosphorus compounds present in soils; some investigators doubt that those listed account for all the organic phosphorus.

17:5. FACTORS THAT CONTROL THE AVAILABILITY OF INORGANIC SOIL PHOSPHORUS

The availability of inorganic phosphorus is largely determined by the following factors: (a) soil pH; (b) soluble iron, aluminum, and manganese; (c) presence of iron-, aluminum-, and manganese-containing minerals; (d) available calcium and calcium minerals; (e) amount and decomposition of organic matter; and (f) activities of microorganisms. The first four factors are interrelated because their effects are largely dependent upon soil pH.

17:6. pH AND PHOSPHATE IONS

As indicated in Chapter 14 (pp. 389–90), the availability of phosphorus to plants is determined to no small degree by the ionic form of this element

[2] Phytin is a calcium–magnesium salt of inositol phosphoric acid and is rather widely distributed in plants, especially in the seeds. Nucleic acids are found in both plants and animals and are even more complex than is phytin. They are apparently polymeric combinations of phosphoric acid, carbohydrates, and bases such as pyrimidine and purine.

(see Fig. 17:2). The ionic form in turn is determined by the pH of the solution in which the ion is found. Thus, in highly acid solutions only the $H_2PO_4^-$ ions are present. If the pH is increased, first the HPO_4^{--} ions and finally PO_4^{3-} ions dominate. This situation is shown by the following equations:

$$\underset{\text{(Very acid solutions)}}{H_2PO_4^-} \overset{OH}{\rightleftharpoons} H_2O + HPO_4^{--} \overset{OH}{\rightleftharpoons} H_2O + \underset{\text{(Very alkaline solutions)}}{PO_4^{3-}}$$

At intermediate pH levels two of the phosphate ions may be present simultaneously. Thus, in solutions at pH 6.0, both $H_2PO_4^-$ and HPO_4^{--} ions are found.

In general, the $H_2PO_4^-$ ion is considered somewhat more available to plants than is the HPO_4^{--} ion. In soils, however, this relationship is complicated by the presence or absence of other compounds or ions. For example, the presence of soluble iron and aluminum under very acid conditions, or calcium at high pH values, will markedly affect the availability of the phosphorus. Clearly, therefore, the effect of soil pH on phosphorus availability is determined in no small degree by the various cations present. The effect of these ions in acid soils will be discussed first.

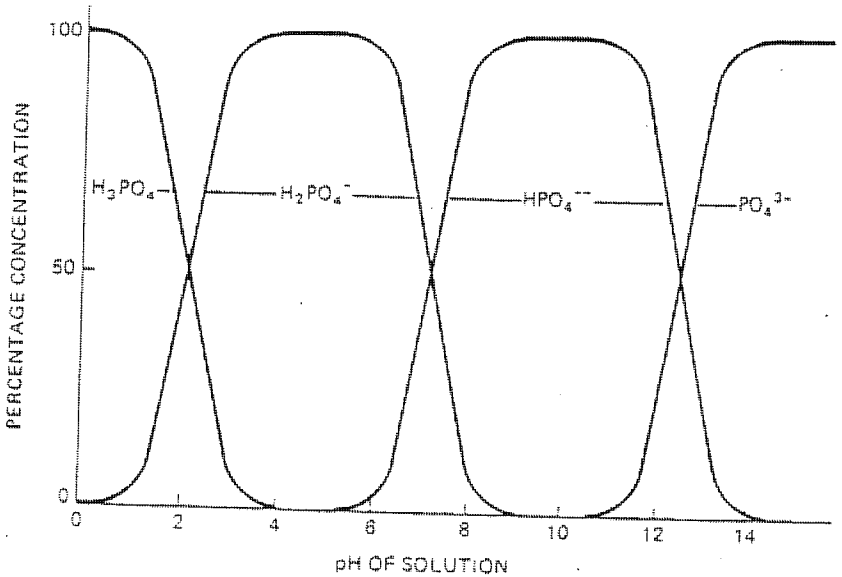


FIGURE 17:2. Relationship between solution pH and the relative concentrations of three soluble forms of phosphate. In the pH range common for soils, the $H_2PO_4^-$ ions predominate.

17:7. Inorganic Phosphorus Availability in Acid Soils

Assume that there is either a nutrient solution or an organic soil very low in inorganic matter (see Fig. 17:3). Assume also that these media are acid in reaction but that they are low in iron, aluminum, and manganese. The $H_2PO_4^-$ ions, which would dominate under these conditions, would be readily available for plant growth. Normal phosphate absorption by plants would be expected so long as the pH was not too low.

PRECIPITATION BY IRON, ALUMINUM, AND MANGANESE IONS. If the same degree of acidity should exist in a normal mineral soil, however, quite different results would be expected. Some soluble iron, aluminum, and manganese are usually found in strongly acid mineral soils. Reaction with the $H_2PO_4^-$ ions would immediately occur, rendering the phosphorus insoluble and also unavailable for plant growth.

The chemical reactions occurring between the soluble iron and aluminum and the $H_2PO_4^-$ ions probably result in the formation of hydroxy phosphates (15). This may be represented as follows, using the aluminum cation as an example:

$$Al^{3+} + H_2PO_4^- + 2H_2O \rightleftharpoons 2H^+ + Al(OH)_2H_2PO_4$$

(Soluble) (Insoluble)

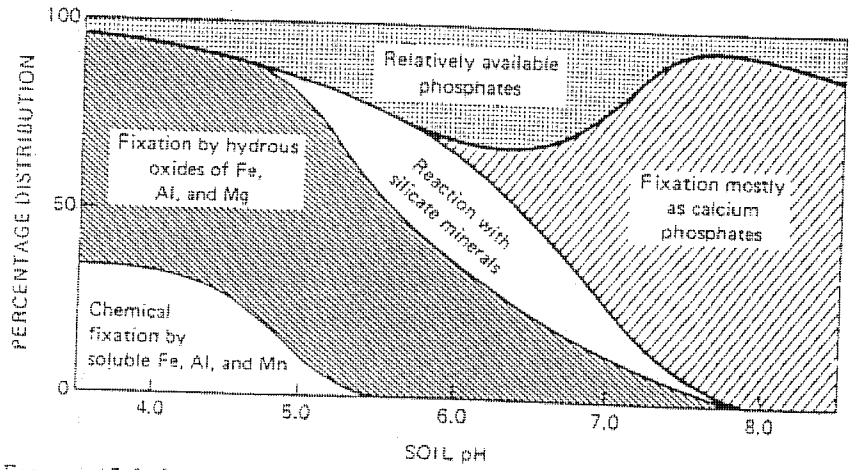


FIGURE 17:3. Inorganic fixation of added phosphates at various soil pH values. Average conditions are postulated and it is not to be inferred that any particular soil would have exactly the same distribution. The actual proportion remaining in an available form will depend upon contact with the soil, time for reaction, and other factors. It should be kept in mind that some of the added phosphorus may be changed to an organic form in which it would be temporarily unavailable.

In most strongly acid soils the concentration of the iron and aluminum ions greatly exceeds that of the $H_2PO_4^-$ ions. Consequently, the above reaction moves to the right, forming the insoluble phosphate. This leaves only minute quantities of the $H_2PO_4^-$ ion immediately available for plants under these conditions.

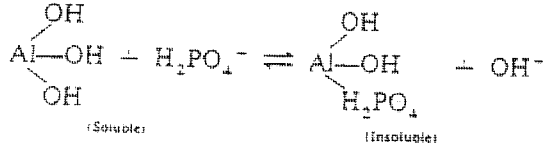
An interesting series of reactions occur when fertilizers containing $Ca(H_2PO_4)_2$ are added to soils, even those relatively high in pH (18) (see Fig. 17:4). The $Ca(H_2PO_4)_2$ in the fertilizer granules attracts water from the soil and the following reaction occurs:

$$Ca(H_2PO_4)_2 \cdot H_2O + H_2O \longrightarrow CaHPO_4 \cdot 2H_2O + H_3PO_4$$

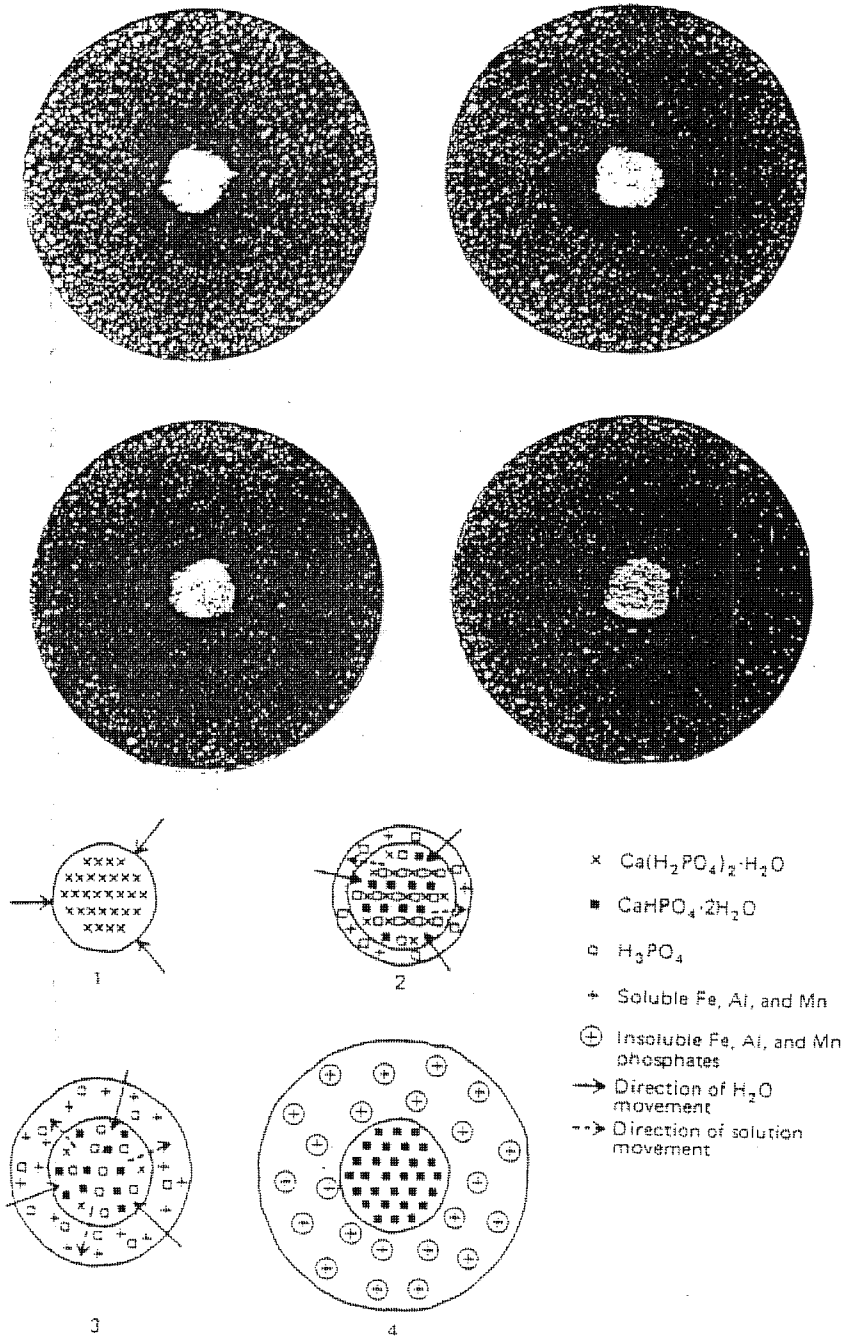
As more water is attracted, a H_3PO_4-laden solution with a pH of about 1.4 moves outward from the granule. This solution is sufficiently acid to dissolve and displace large quantities of iron, aluminum, and manganese. These ions react with the phosphate to form complex compounds, which later probably revert to the hydroxy phosphates of iron, aluminum, and manganese in acid soils and of calcium in neutral to alkaline soils. In any case, the immediate products of the addition to soils of a water-soluble compound $[Ca(H_2PO_4)_2 \cdot H_2O]$ are a group of insoluble iron, aluminum, manganese, and calcium compounds. Even so, the phosphorus in these compounds is released quite readily for plant growth. It is only after these freshly precipitated compounds are allowed to "age" or to revert to more insoluble forms that availability to plants is greatly reduced.

FIXATION BY HYDROUS OXIDES. It should be emphasized that the $H_2PO_4^-$ ion reacts not only with the soluble iron, aluminum, and manganese but also with insoluble hydrous oxides of these elements, such as limonite and goethite. The actual quantity of phosphorus fixed by these minerals in acid soils quite likely exceeds that due to chemical precipitation by the soluble iron, aluminum, and manganese cations (see Fig. 17:3).

The compounds formed as a result of fixation by iron and aluminum oxides are likely to be hydroxy phosphates, just as in the case of chemical precipitation described above (15). Their formation can be illustrated by means of the following equation if the hydrous oxide of aluminum is represented as aluminum hydroxide:



By means of this and similar reactions the formation of several basic phosphate minerals containing either iron or aluminum or both is thought to occur. Since several such compounds are possible, fixation of phosphorus by this mechanism probably takes place over a relatively wide pH range.



Also the large quantities of hydrous iron and aluminum oxides present in most soils make possible the fixation of tremendous total amounts of phosphorus by this means.

Thus, as both of the equations above show, the acid condition which would make possible the presence of the readily available $H_2PO_4^-$ ion in mineral soils at the same time results in conditions conducive to the vigorous *fixation* or precipitation of the phosphorus by iron, aluminum, and manganese compounds (see Fig. 17:4).

FIXATION BY SILICATE CLAYS. A third means of fixation of phosphorus under moderately acid conditions involves silicate minerals such as kaolinite, montmorillonite, and illite. Although there is some doubt about the actual mechanisms involved, the overall effect is essentially the same as when phosphorus is fixed by simpler iron and aluminum compounds. Some scientists visualize the fixation of phosphates by silicate minerals as a surface reaction between exposed —OH groups on the mineral crystal and the $H_2PO_4^-$ ions. Other investigators have evidence that aluminum and iron ions are removed from the edges of the silicate crystals forming hydroxy phosphates of the same general formula as those already discussed. This type of reaction might be expressed as follows:

$$\underset{\text{(in silicate crystal)}}{[Al]} + H_2PO_4^- + 2H_2O \rightleftharpoons 2H^- + \underset{\text{(insoluble)}}{Al(OH)_2H_2PO_4}$$

Thus, even though phosphates react with different ions and compounds in acid soils, apparently the same insoluble iron and aluminum compounds are formed in each case. Major differences from soil to soil are probably due to differences in rate of phosphate precipitation and in the surface area of the phosphates once the reaction has occurred. This will be discussed again later.

ANION EXCHANGE. Part of the phosphate which has reacted with iron and aluminum compounds and with silicate clays is subject to replacement by other anions, such as the hydroxyl ion. Such replacement is called *anion exchange*. It may be illustrated by a reverse of the reaction on page 463.

FIGURE 17:4 [OPPOSITE]. Reaction of $Ca(H_2PO_4)_2 \cdot H_2O$ granules with moist soil. (1) The granule has just been added to the soil and is beginning to absorb water from it. (2) In the moistened granule H_3PO_4 and $CaHPO_4 \cdot 2H_2O$ are being formed and more soil water is being absorbed. (3) The H_3PO_4-laden solution moves into the soil, dissolving and displacing Fe, Al, and Mn and leaving insoluble $CaHPO_4 \cdot 2H_2O$ in the granule. (4) The Fe, Al, and Mn ions have reacted with the phosphate to form insoluble compounds, which, along with the residue of $CaHPO_4 \cdot 2H_2O$, are the primary reaction products. [*Photos courtesy G. I. Terman and National Plant Food Institute, Washington, D.C.*]

Thus,

$$Al(OH)_2H_2PO_4 + OH^- \rightleftharpoons Al(OH)_3 + H_2PO_4^-$$

One anion (OH) has been exchanged for another (H_2PO_4). This reaction shows how anion exchange can take place and illustrates the importance of liming in helping to maintain a higher level of available phosphates.

17:8. Inorganic Phosphorus Availability at High pH Values

In alkaline soils, phosphate precipitation is caused mostly by calcium compounds (see Fig. 17:3). Such soils are plentifully supplied with exchangeable calcium and in most cases with calcium carbonate. Available phosphates will react with both the calcium ion and its carbonate. As an illustration, assume that concentrated superphosphate is added to a calcareous soil. The reactions would be as follows:

$$\underset{\text{(Soluble)}}{Ca(H_2PO_4)_2} + \underset{\text{(Adsorbed)}}{2Ca^{++}} \rightleftharpoons \underset{\text{(Insoluble)}}{Ca_3(PO_4)_2} + 4H^+$$

$$\underset{\text{(Soluble)}}{Ca(H_2PO_4)_2} + 2CaCO_3 \rightleftharpoons \underset{\text{(Insoluble)}}{Ca_3(PO_4)_2} + 2CO_2\uparrow + 2H_2O$$

Although the $Ca_3(PO_4)_2$ thus formed is quite insoluble, it may be converted in the soil to even more insoluble compounds. Hydroxy, oxy, carbonate, and even fluor apatite compounds may be formed if conditions are favorable and if sufficient time is allowed (see Table 17:3).

This type of reversion may occur in soils of the eastern United States which have been heavily limed. It is much more serious, however, in Western soils, owing to the widespread presence of excess $CaCO_3$. The problem of utilizing phosphates in alkaline soils of the arid West is thus fully as serious as it is on highly acid soils in the East.

17:9. pH for Maximum Inorganic Phosphorus Availability

With insolubility of phosphorus occurring at both extremes of the soil pH range (see Fig. 17:3), the question arises as to the range in soil reaction in which minimum fixation occurs. The basic iron and aluminum phosphates have a minimum solubility around pH 3 to 4. At higher pH values some of the phosphorus is released and the fixing capacity somewhat reduced. Even at pH 6.5, however, much of the phosphorus is still probably chemically combined with iron and aluminum. As the pH approaches 6, precipitation as calcium compounds begins; at pH 6.5 the formation of insoluble calcium salts is a factor in rendering the phosphorus unavailable. Above pH 7.0, even more insoluble compounds, such as apatites, are formed.

These facts seem to indicate that maximum phosphate availability to plants is obtained when the soil pH is maintained in the range from 6.0 to 7.0 (see Fig. 17:3). Even in this range, however, the fact should be emphasized that phosphate availability may still be very low and that added soluble phosphates are readily fixed by soils. The low recovery (perhaps 10 to 30 percent) by plants of added phosphates in a given season is partially due to this fixation.

17:10. Availability and Surface Area of Phosphates

When soluble phosphates are added to soils two kinds of compounds form immediately: (a) fresh precipitates of calcium, or iron and aluminum phosphates; and (b) similar compounds formed on the surfaces of either calcium carbonate or iron and aluminum oxide particles. In each case, the total surface area of the phosphate is high, and consequently the availability of the phosphorus contained therein is reasonably rapid. Thus, even though the water-soluble phosphorus in superphosphate may be precipitated in the soil in a matter of a few days, the freshly precipitated compounds will release much of their phosphorus to growing plants.

EFFECTS OF AGING. With time, changes take place in the reaction products of soluble phosphates and soils. These changes generally result in a reduction in surface area of the phosphates and a similar reduction in their availability. An increase in the crystal size of precipitated phosphates occurs in time. This decreases their surface area. Also, there is a penetration of the phosphorus held by calcium carbonate and iron or aluminum oxide particles into the particle itself (see Fig. 17:5). This leaves less of the phosphorus near the surface where it can be made available to growing plants. By these

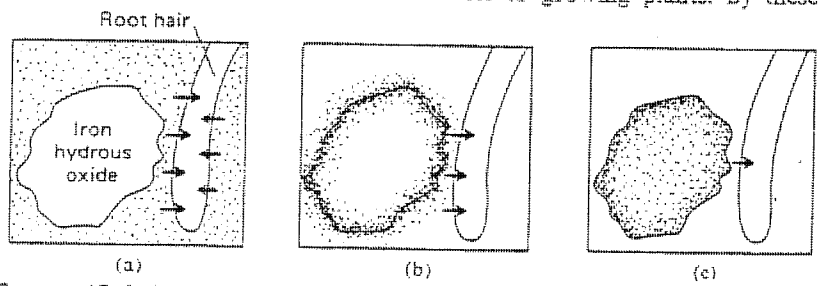


FIGURE 17:5. How relatively soluble phosphates are rendered unavailable by compounds such as hydrous oxide. (a) The situation just after application of a soluble phosphate. The root hair and the hydrous iron oxide particle are surrounded by soluble phosphates. Within a very short time (b) most of the soluble phosphate has reacted with the surface of the iron oxide crystal. The phosphorus is still fairly readily available to the plant roots since most of it is located at the surface of the particle where exudates from the plant can encourage exchange. In time (c) the phosphorus penetrates the crystal and only a small portion is found near the surface. Under these conditions its availability is low.

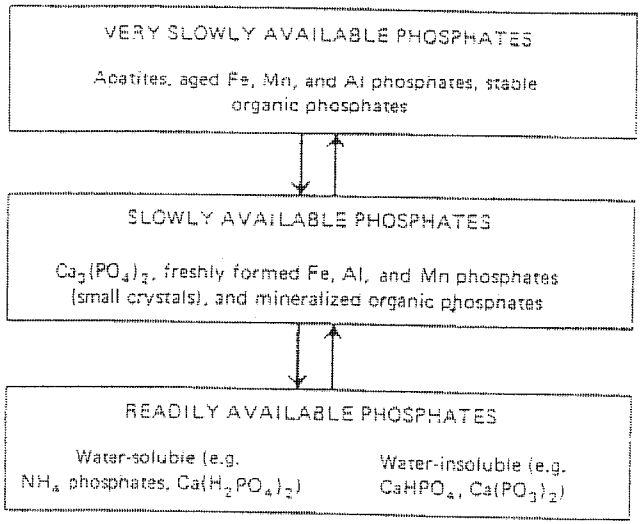


FIGURE 17:6. Classification of phosphate compounds in three major groups. Fertilizer phosphates are generally in the "readily available phosphate" group but are quickly converted to the slowly available forms. These can be utilized by plants at first but upon aging are rendered less available and are then classed as very slowly available. At any one time perhaps 80–90 percent of the soil phosphorus is in "very slowly available" forms. Most of the remainder is in the slowly available form since perhaps less than 1 percent would be expected to be readily available.

processes of aging, phosphate availability is reduced. Thus, the supply of available phosphorus to plants is determined not only by the kinds of compounds which form but also by their surface areas (see Fig. 17:6).

17:11. Phosphorus-Fixing Power of Soils

In light of the above discussion it is interesting to note the actual quantity of phosphorus which soils are capable of fixing. Data from three New Jersey soils presented in Table 17:4 emphasize the tremendous power of certain soils in this respect. For example, to satisfy the phosphorus-fixing power of the unlimed Collington soil, nearly 47 tons of superphosphate containing 20 percent P_2O_5 would be required. Although liming definitely reduced the fixing capacity, the quantity of phosphorus fixed even on the limed soils is enormous. Thus, over 25 tons of superphosphate would be required to completely satisfy the phosphorus-fixing power of the limed Collington soil.

One Coastal Plain soil was reported (4) to have a phosphate-fixing capacity of 125 tons of 20 percent superphosphate per acre-furrow slice. Although such values are somewhat higher than usual because of the nature and amounts of the iron and aluminum compounds in the soils, they do not overemphasize the problem of phosphate fixation.

TABLE 17:4. *Phosphorus-Adsorbing Power of Three New Jersey Soils Limed and Unlimed*[a]

Expressed as pounds of 20 percent superphosphate per acre-furrow slice.

Soil	Treatment	pH	Phosphorus Fixing Power (lb 20% super/A.F.S.)
Sassafras	No lime	3.6	28,400
Sassafras	Lime	6.5	13,916
Collington	No lime	3.2	93,720
Collington	Lime	6.5	50,268
Dutchess	No lime	3.8	68,728
Dutchess	Lime	6.5	44,020

[a] From Toth and Bear (24).

17:12. Influence of Soil Organisms and Organic Matter on the Availability of Inorganic Phosphorus

In addition to pH and related factors, organic matter and microorganisms strikingly affect inorganic phosphorus availability. Just as was the case with nitrogen, the rapid decomposition of organic matter and consequent high microbial population results in the *temporary* tying up of inorganic phosphates in microbial tissue.

Products of organic decay such as organic acids and humus are thought to be effective in forming complexes with iron and aluminum compounds. This engagement of iron and aluminum reduces inorganic phosphate fixation to a remarkable degree. The exact importance of this effect has not as yet been completely ascertained. The ability of humus and lignin to reduce phosphate fixation, however, is shown in Fig. 17:7. Both materials were effective in releasing phosphorus after it had been fixed as basic iron phosphate. Thus, organic decomposition products undoubtedly play an important role in organic phosphorus availability.

17:13. Availability of Organic Phosphorus

Only meager information has been obtained on the factors affecting the availability to higher plants or organic phosphorus compounds. It has been established that both *phytin* and *nucleic acids* can be utilized as sources of phosphorus (20). Apparently the phytin is absorbed directly by the plants while the nucleic acids probably are broken down by enzymes at the root surfaces and the phosphorus is adsorbed in either the organic or inorganic form. In spite of the readiness with which these compounds may be assimilated, however, plants commonly suffer from a phosphorus deficiency even in the presence of considerable quantities of organic forms of this element. Just as with inorganic phosphates, the problem is one of availability.

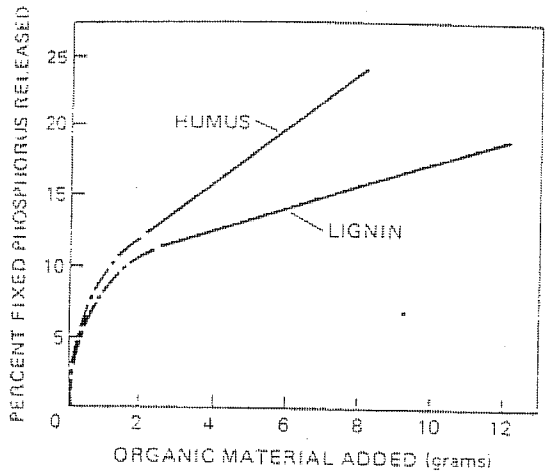


FIGURE 17:7. Effect of added organic materials on the release of phosphorus previously fixed by iron compounds. Both humus and lignin are effective, the humus to the greater degree. These results suggest that mineral fixation of phosphorus may be lower in soils comparatively high in organic matter. [*After Swenson et al.* (22).]

Phytin behaves in the soil much as do the inorganic phosphates (8), forming iron, aluminum, and calcium phytates. In acid soils the phytin is rendered insoluble and thus unavailable because of reaction with iron and aluminum. Under alkaline conditions calcium phytate is precipitated and the phosphorus carried is rendered unavailable.

The fixation of nucleic acids involves an entirely different mechanism, but the end result—low phosphorus availability—is the same. Evidently, nucleic acids are strongly adsorbed by clays, especially montmorillonite.

This adsorption is particularly pronounced under acid conditions and results in a marked decrease in the rate of decomposition of the nucleic acids. Consequently, the available phosphorus supply from this source is low, especially in acid soils which contain appreciable amounts of montmorillonite.

The judicious application of lime to acid soils is thus fully as important in organic phosphorus nutrition as it is in rendering inorganic compounds available. Whether we are dealing with inorganic soil phosphates, added fertilizers, or organic materials, the importance of lime as a controlling factor in phosphate availability is clearly evident.

17:14. Practical Control of Phosphorus Availability

From a practical standpoint, the phosphorus-utilization picture is not too encouraging. The inefficient utilization of applied phosphates by plants has long been known. The experimental use of radioactive phosphorus materials has emphasized this point even more thoroughly. By adding fertilizers containing traceable phosphorus, it has been possible to determine the proportion of the applied phosphates absorbed during the year of application.

The results of experiments on corn, soybeans, and potatoes are shown in Table 17:5. Even though on some soils marked responses were obtained from the addition of phosphate fertilizers, the efficiency of phosphorus utilization was very low. Apparently maximum yields were obtained only by supplying much more phosphorus than the plants absorbed in a given season.

TABLE 17:5. *Recovery of Applied Fertilizer Phosphates During the First Crop Year*

Soil	Crop	Fertilizer Phosphorus Recovered the First Year (%)
Bladen	Corn	11.3
Bladen	Potatoes	7.6
Bladen	Soybeans	18.2
Webster	Corn	6.5
Clarion	Corn	3.4

[a] Data for bladen soil from Krantz, et al. (16) and for other soils from Stanford and Nelson (21).

LIMING AND PLACEMENT OF FERTILIZERS. The small amount of control that can be exerted over phosphate availability seems to be associated with *liming*, *fertilizer placement*, and *organic matter maintenance*. By holding the pH of soils between 6.0 and 7.0, the phosphate fixation can be kept at a minimum (see Figs. 14:7 and 17:2). In order to prevent rapid reaction of phosphate fertilizers with the soil, these materials are commonly placed in localized bands. In addition, phosphatic fertilizers are quite often pelleted or aggregated to retard still more their contact with the soil. The effective utilization of phosphorus in combination with animal manures is evidence of the importance of organic matter in increasing the availability of this element.

In spite of these precautions, a major portion of the added phosphates still reverts to less available forms (see Fig. 17:8). It should be remembered, however, that the reverted phosphorus is not lost from the soil and through the years undoubtedly is slowly available to growing plants. This becomes an important factor, especially in soils which have been heavily phosphated for years.

In summary, maintaining sufficient available phosphorus in a soil largely narrows down to a twofold program: (a) the addition of phosphorus-containing fertilizers, and (b) the regulation in some degree of the fixation in the soil of both the added and the native phosphates.

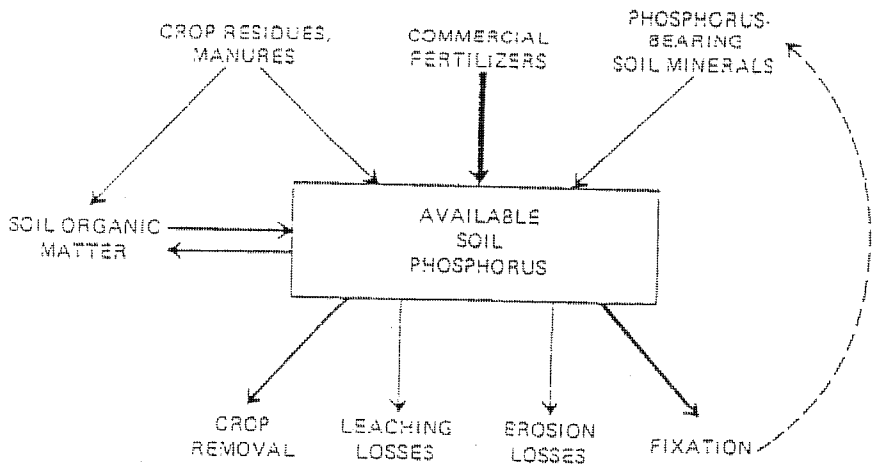


FIGURE 17:8. How the available phosphorus level in a soil is depleted and replenished. Note that the two main features are the addition of phosphate fertilizers and the fixation of this element in insoluble forms. It should be remembered that the amount of available phosphorus in the soil at any one time is relatively small, especially when compared to that of calcium, magnesium, and potassium.

17:15. POTASSIUM—THE THIRD "FERTILIZER" ELEMENT

The history of fertilizer usage in the United States shows that nitrogen and phosphorus received most of the attention when commercial fertilizers first appeared on the market. Although the role played by potassium in plant nutrition has long been known, the importance of potash fertilization has received full recognition only in comparatively recent years.

The reasons that a widespread deficiency of this element did not develop earlier are at least twofold. First, the supply of available potassium originally was so high in most soils that it took many years of cropping for a serious depletion to appear. Second, even though the potassium in certain soils may have been insufficient for optimum crop yields, production was much more drastically limited by a lack of nitrogen and phosphorus. With an increased usage of fertilizers carrying these latter elements, crop yields have been correspondingly increased. As a consequence, the drain on soil potassium has been greatly increased. This, coupled with considerable loss by leaching, has enhanced the demand for potassium in commercial fertilizers.

17:16. EFFECTS OF POTASSIUM ON PLANT GROWTH

The presence of adequate available potassium in the soil has much to do with the general tone and vigor of the plants grown. Moreover, by increasing

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TABLE 2:2. *Range in Micronutrient Content Commonly Found in Soils and a Suggested Analysis of a Representative Surface Soil*

Nutrient	Normal Range		Suggested Analysis of a Representative Surface Soil (ppm)
	Percent	ppm[a]	
Iron	0.500 –5.000	5,000–50,000	25,000
Manganese	0.020 –1.000	200–10,000	2,500
Zinc	0.001 –0.025	10– 250	100
Boron	0.0005 –0.015	5– 150	50
Copper	0.0005 –0.015	5– 150	50
Chlorine	0.001 –0.1	10– 1,000	50
Cobalt	0.0001 –0.005	1– 50	15
Molybdenum	0.00002–0.0005	0.2– 5	2

[a] ppm = parts per million. These estimates are based on published data from a number of sources, especially Mitchell (4).

The deficiencies of micronutrients discovered in many of our soils in recent years have highlighted the practical significance of these elements. Because macronutrient fertility problems are more widespread, however, they will be given our first attention. Micronutrients will be considered in more detail in Chapter 18.

FOUR NUTRIENT QUESTIONS. To arrive at a logical conclusion as to why nutrient deficiencies often occur in soils, four phases must be examined. They are (a) the macronutrient contents of mineral soils, (b) their forms of combination, (c) the processes by which these elements become available to plants, and (d) the soil solution and its pH. These phases will be considered in order.

2:5. MACRONUTRIENT CONTENTS OF MINERAL SOILS

The chemical composition for representative surface soils of humid temperate and arid temperate regions is given in Table 2:3. It should be noted that such figures do not fit any particular soil but present a very rough average of the data available for top soils of these two regions. These data suggest that soils of arid regions are in general higher in all of the important constituents except organic matter and nitrogen. An exception even to this is found in the black earth soils (Mollisols) of subhumid regions, which sometimes range as high as 16 percent of organic matter and 0.70 to 0.80 percent of nitrogen.

ORGANIC MATTER, NITROGEN, AND PHOSPHORUS. The percentage of organic matter exceeds that of any other constituent listed in Table 2:3. Yet its

TABLE 2:3. *Total Amounts of Organic Matter and Primary Nutrients Present in Temperate Region Mineral Surface Soils*[a]

Constituents	Ranges That Ordinarily May Be Expected (%)	Representative Analyses: Humid Region Soil, Percent	Humid Region Soil, lb/acre-furrow slice	Arid Region Soil, Percent	Arid Region Soil, lb/acre-furrow slice[b]
Organic matter	0.40–10.00	4.00	80,000	3.25	65,000
Nitrogen (N)	0.02– 0.50	0.15	3,000	0.12	2,400
Phosphorus (P)	0.01– 0.20	0.04	800	0.07	1,400
Potassium (K)	0.17– 3.30	1.70	34,000	2.00	40,000
Calcium (Ca)	0.07– 3.60	0.40	8,000	1.00	20,000
Magnesium (Mg)	0.12– 1.50	0.30	6,000	0.60	12,000
Sulfur (S)	0.01– 0.20	0.04	800	0.08	1,600

[a] As a supplement to the generalized figures of Table 2:3 the analyses of eight representative United States surface soils are presented as published by Marbut (3):

Constituents	Norfolk Fine Sand, Florida (%)	Sassafras Sandy Loam, Virginia (%)	Ontario Loam, New York (%)	Loam from Ely, Nevada (%)	Hagerstown Silt Loam, Tennessee (%)	Cascade Silt Loam, Oregon (%)	Marshall Silt Loam, Iowa (%)	Summit Clay from Kansas (%)
SiO_2	91.49	85.96	76.54	61.69	73.11	70.40	72.63	71.60
TiO_2	0.50	0.59	0.64	0.47	1.05	1.08	0.63	0.81
Fe_2O_3	1.75	1.74	3.43	3.87	6.12	3.90	3.14	3.56
Al_2O_3	4.51	6.26	9.38	13.77	8.30	13.14	12.03	11.45
MnO	0.007	0.04	0.08	0.12	0.44	0.07	0.10	0.06
CaO	0.01	0.40	0.80	5.48	0.37	1.78	0.79	0.97
MgO	0.02	0.36	0.75	2.60	0.45	0.97	0.82	0.86
K_2O	0.16	1.54	1.95	2.90	0.91	2.11	2.23	2.42
Na_2O	Trace	0.58	1.04	1.47	0.20	1.98	1.36	1.04
P_2O_5	0.05	0.02	0.10	0.18	0.16	0.16	0.12	0.09
SO_3	0.05	0.07	0.08	0.12	0.07	0.21	0.12	0.11
Nitrogen	0.02	0.02	0.16	0.10	0.27	0.08	0.17	0.09

[b] The furrow slice of a representative mineral soil is considered to contain approximately 2 million pounds of dry earth to the acre.

amount in most surface soils usually is critical. It is of prime importance in keeping the soil loose and open and is an essential source of several nutrient elements. The addition and subsequent decay of organic matter in the soil is thus highly significant both physically and chemically.

Nitrogen and phosphorus are almost always present in comparatively small amounts in mineral soils. Moreover, a large proportion of these elements at any one time is held in combinations unavailable to plants. For example, even the more simple compounds of phosphorus are relatively

insoluble in most soils. As a result, this element is doubly critical—low total amounts and very low availability to plants.

POTASSIUM, CALCIUM, AND MAGNESIUM. The total quantity of potassium, in marked contrast to phosphorus, is usually plentiful except in sandy soils. The main problem is one of availability. Calcium shows great variation but it is generally present in lesser amounts than is potassium. When it is lacking, soils tend to be acid. Calcium-containing limestones are generally added to correct this condition.

Magnesium, besides its importance as a nutrient, functions in the soil much as does calcium. Its deficiency in some soils has long been suspected. Until recently, however, it has not been considered especially critical because it is carried by most limestones, sometimes in large amounts. Where liming[1] is practiced, the lack of magnesium often is automatically rectified. In spite of this, magnesium deficiency is a major problem in many areas in the eastern United States.

SULFUR. Although it is usually no more plentiful than phosphorus, sulfur is more readily available. This is because its simple inorganic compounds are not rendered insoluble by reacting with certain other soil constituents as is the case with phosphorus. As already suggested, the addition of sulfur in farm manure, rain water, and fertilizers tends in an automatic way to relieve a possible deficiency in humid temperate regions. In certain areas of the west and south, however, specific additions of sulfur-containing compounds are required.

CRITICAL CONSTITUENTS. The above discussion seems to indicate that three constituents are likely to be critical in almost all mineral soils. Two—*organic matter* and *nitrogen*—merit particular attention because of the small amounts originally present and because of their ready loss through oxidation, leaching, or crop removal. The third, *phosphorus*, faces a double handicap as already explained—an exceptionally small amount present and a low availability to higher plants.

Under humid conditions *calcium* by all means must be included in the above list because it is sure to be much depleted by leaching. Consequently, it is needed not only as a nutrient but also as a means of controlling soil acidity. In arid regions, where the leaching of calcium usually is negligible, this nutrient is likely to be present in abundance, especially in the subsoil.

[1] Liming refers to the application to agricultural soils of basic calcium- and magnesium-containing materials with the objective of reducing soil acidity. Ground limestones are most commonly used, although burned lime (CaO) or slaked lime [$Ca(OH)_2$] are sometimes added. Collectively, these materials are referred to as *lime* and the practice of adding them as *liming* (see Chapter 15).

17:2. Influence of Phosphorus on Plants

It is difficult to state in detail the functions of phosphorus in the economy of even the simplest plants. Only the more important functions will be considered here. Phosphorus makes its contribution through its favorable effect on the following:

1. Cell division and fat and albumin formation.
2. Flowering and fruiting, including seed formation.
3. Crop maturation, thus counteracting the effects of excess nitrogen applications.
4. Root development, particularly of the lateral and fibrous rootlets.
5. Strength of straw in cereal crops, thus helping to prevent lodging.
6. Crop quality, especially of forages and of vegetables.
7. Resistance to certain diseases.

17:3. The Phosphorus Problem

Although the amount of total phosphorus in an average mineral soil compares favorably with that of nitrogen, it is much lower than potassium, calcium, or magnesium (see Table 2:3). Of even greater importance, however, is the fact that most of the phosphorus present in soils is currently unavailable to plants. Also, when soluble sources of this element are supplied to soils in the form of fertilizers, their phosphorus is often "fixed" or rendered insoluble or unavailable to higher plants, even under the most ideal field conditions (see p. 465).

Fertilizer practices in many areas exemplify the problem of phosphorus availability. As already emphasized, the tonnage of phosphorus-supplying materials used as fertilizers definitely exceeds all except the nitrogen carriers. The removal of phosphorus from soils by crops, however, is low compared to that of nitrogen and potassium, often being only one third or one fourth that of the latter elements. The necessity for high fertilizer dosage when relatively small quantities of phosphorus are being removed from soils indicates that much of the added phosphates becomes unavailable to growing plants.

The influence of this situation on fertilizer practice is clearly shown when considering the additions of fertilizer phosphorus in comparison with crop removal.

In the United States, phosphorus added in fertilizers exceeds that removed by crops by more than 24 percent (see Table 17:1). In some areas, notably the eastern seaboard states, additions of phosphorus more than triple the removal of this element by crops. Since phosphorus is lost only sparingly by leaching, the inefficiency of utilization of phosphate fertilizers is obvious.

Briefly, then, the overall phosphorus problem is threefold: (a) a small total amount present in soils, (b) the unavailability of such native phosphorus, and (c) a marked "fixation" of added soluble phosphates. Since crop removal

of phosphorus is relatively low and world phosphate supplies are huge, problem (a), that of supplying sufficient total phosphorus, is not serious. Increasing the availability of native soil phosphorus and the retardation of fixation or reversion of added phosphates are, therefore, the problems of greatest importance. These two phases will be discussed following a brief review of the phosphorus compounds present in soils.

TABLE 17:1. *Nutrients Removed by Crops in the United States Compared to That Added in Fertilizers* (1965)[a]

	N	P	K
Removed in crops (thousands of tons)	8,838	1,207	4,152
Added in fertilizers (thousands of tons)	4,580	1,499	2,313
Addition as percent of removal	52	124	56

[a] Nutrient removal figures calculated from White (25) Fertilizer additions from Tennessee Valley Authority (23).

17:4. PHOSPHORUS COMPOUNDS IN SOILS[1]

Both inorganic and organic forms of phosphorus occur in soils and both are important to plants as sources of this element. There is a serious lack of information, however, on the relative amounts of these two forms in different soils. Data available from Oregon, Iowa, and Arizona (Table 17:2) give some idea of their relative proportions. Despite the variation which occurs, it is evident that a consideration of soil phosphorus would not be complete unless some attention were given to both forms (see Fig. 17:1).

INORGANIC COMPOUNDS. Most inorganic phosphorus compounds in soils fall into one of two groups: (a) those containing *calcium*, and (b) those containing *iron* and *aluminum*. The calcium compounds of most importance are listed in Table 17:3. Fluorapatite, the most insoluble and unavailable of the group, usually is an original mineral. It is found in even the more weathered soils, especially in their lower horizons. This fact is an indication of the extreme insolubility and consequent unavailability of the phosphorus contained therein. The simpler compounds of calcium, such as mono and dicalcium phosphate, are readily available for plant growth. Except on recently fertilized soils, however, these compounds are present in extremely small quantities only since they easily revert to the more insoluble forms.

Much less is known of the exact constitution of the iron and aluminum phosphates contained in soils. The compounds involved are probably hydroxy phosphates such as dufrenite, wavellite, strengite, and variscite (10). These compounds are most stable in acid soils and are extremely insoluble.

[1] For a review of soil phosphorus, see Larsen (17).

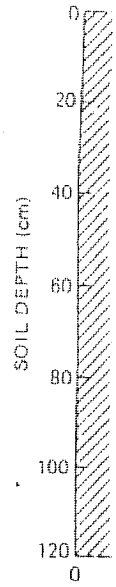

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TABLE 17:2. *Ton*
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[a] Figures for Oregon fro
for Arizona from Full

plants is obtained when the soil pH is maintained in the range from 6.0 to 7.0 (see Fig. 17:3). Even in this range, however, the fact should be emphasized that phosphate availability may still be very low and that added soluble phosphates are readily fixed by soils. The low recovery (perhaps 10 to 30 percent) by plants of added phosphates in a given season is partially due to this fixation.

17:10. Availability and Surface Area of Phosphates

When soluble phosphates are added to soils two kinds of compounds form immediately: (a) fresh precipitates of calcium, or iron and aluminum phosphates; and (b) similar compounds formed on the surfaces of either calcium carbonate or iron and aluminum oxide particles. In each case, the total surface area of the phosphate is high, and consequently the availability of the phosphorus contained therein is reasonably rapid. Thus, even though the water-soluble phosphorus in superphosphate may be precipitated in the soil in a matter of a few days, the freshly precipitated compounds will release much of their phosphorus to growing plants.

EFFECTS OF AGING. With time, changes take place in the reaction products of soluble phosphates and soils. These changes generally result in a reduction in surface area of the phosphates and a similar reduction in their availability. An increase in the crystal size of precipitated phosphates occurs in time. This decreases their surface area. Also, there is a penetration of the phosphorus held by calcium carbonate and iron or aluminum oxide particles into the particle itself (see Fig. 17:5). This leaves less of the phosphorus near the surface where it can be made available to growing plants. By these

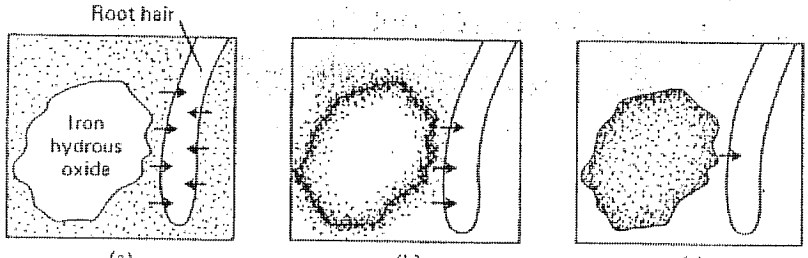

FIGURE 17:5. How relatively soluble phosphates are rendered unavailable by compounds such as hydrous oxide. (a) The situation just after application of a soluble phosphate. The root hair and the hydrous iron oxide particle are surrounded by soluble phosphates. Within a very short time (b) most of the soluble phosphate has reacted with the surface of the iron oxide crystal. The phosphorus is still fairly readily available to the plant roots since most of it is located at the surface of the particle where exudates from the plant can encourage exchange. In time (c) the phosphorus penetrates the crystal and only a small portion is found near the surface. Under these conditions its availability is low.

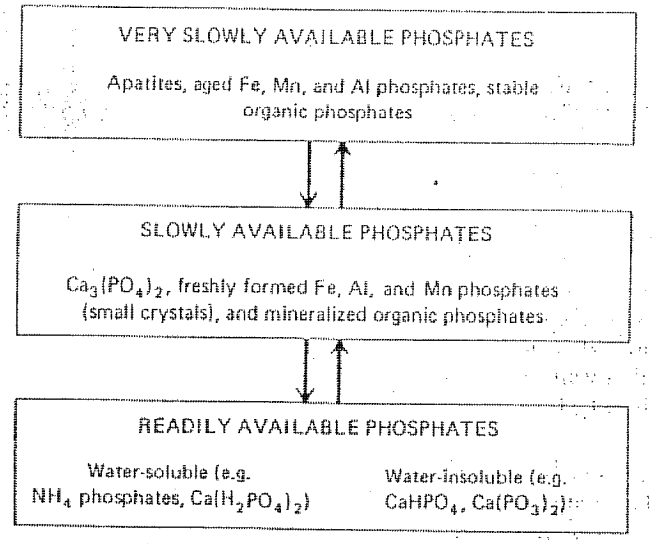

FIGURE 17:6. Classification of phosphate compounds in three major groups. Fertilizer phosphates are generally in the "readily available phosphate" group but are quickly converted to the slowly available forms. These can be utilized by plants at first but upon aging are rendered less available and are then classed as very slowly available. At any one time perhaps 80–90 percent of the soil phosphorus is in "very slowly available" forms. Most of the remainder is in the slowly available form since perhaps less than 1 percent would be expected to be readily available.

processes of aging, phosphate availability is reduced. Thus, the supply of available phosphorus to plants is determined not only by the kinds of compounds which form but also by their surface areas (see Fig. 17:6).

17:11. Phosphorus-Fixing Power of Soils

In light of the above discussion it is interesting to note the actual quantity of phosphorus which soils are capable of fixing. Data from three New Jersey soils presented in Table 17:4 emphasize the tremendous power of certain soils in this respect. For example, to satisfy the phosphorus-fixing power of the unlimed Collington soil, nearly 47 tons of superphosphate containing 20 percent P_2O_5 would be required. Although liming definitely reduced the fixing capacity, the quantity of phosphorus fixed even on the limed soils is enormous. Thus, over 25 tons of superphosphate would be required to completely satisfy the phosphorus-fixing power of the limed Collington soil.

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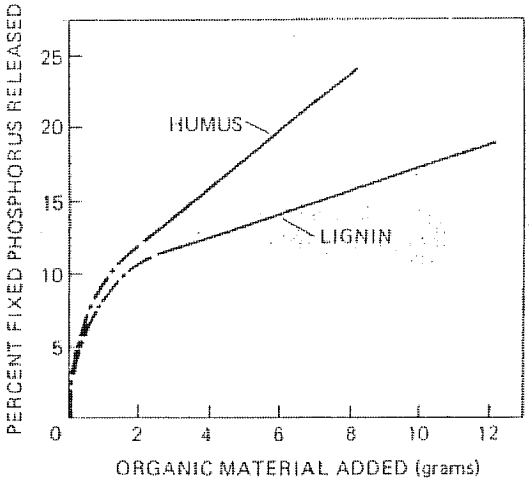

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The fixation of nucleic acids involves an entirely different mechanism, but the end result—low phosphorus availability—is the same. Evidently, nucleic acids are strongly adsorbed by clays, especially montmorillonite.

This adsorption is particularly pronounced under acid conditions and results in a marked decrease in the rate of decomposition of the nucleic acids. Consequently, the available phosphorus supply from this source is low, especially in acid soils which contain appreciable amounts of montmorillonite.

The judicious application of lime to acid soils is thus fully as important in organic phosphorus nutrition as it is in rendering inorganic compounds available. Whether we are dealing with inorganic soil phosphates, added fertilizers, or organic materials, the importance of lime as a controlling factor in phosphate availability is clearly evident.

17:14. PRACTICAL CONTROL OF PHOSPHORUS AVAILABILITY

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TABLE 17:5. *Recovery* [illegible] *App*

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* Data for Bladen soil from Kurtz et

LIMING AND PLACEMENT

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

Amy Plato Roberts
Technical Sciences Group, Inc.
1101 17th Street, NW, Suite 500
Washington, DC 20036

Dear Ms. Roberts:

The Biochemical Classification Committee of the Biopesticides & Pollution Prevention Division (BPPD) has reviewed the information submitted and has determined that the active ingredient Iron (formulated as an Fe-Na-EDTA complex with a carbonate buffer in "flour-based" pellets qualifies to be reviewed in BPPD. As with a another Iron-based product registered by BPPD, your active ingredient/product was classified as "Not a biochemical, but eligible for a reduced data set" and, therefore, reviewable by BPPD. The classification is based on the abundance of Iron in nature, its low toxicity, its use as a nutritional supplement, and its low water solubility. To obtain information regarding how to register a product with U. S. EPA, please visit our web site at www.epa.gov/pesticides. Please go to the section entitled "Registration" to find all of the necessary forms required and information regarding specifics on how to register a pesticide product. There are also cross references found on our "What's New" section which are helpful in developing product labeling. Additional information regarding biopesticides and BPPD may be found at www.epa.gov/oppbppd1/biopesticides/. We recommend that you contact Sheryl Reilly, Biochemical Pesticides Branch Chief (703/308-5071; reilly.sheryl@epa.gov) to arrange a pre-registration meeting.

Should you have any further questions, please feel free to email or call me at the address below.

Sincerely,

Russell S. Jones

Russell S. Jones, Ph.D., Biologist
Chair, Biochemical Classification Committee
Biopesticides & Pollution Prevention Division
Office of Pesticide Programs
U. S. Environmental Protection Agency
Ariel Rios Bldg. (7511C)
1200 Pennsylvania Avenue
Washington, DC 20460
703/308-5071
jones.russell@epa.gov

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SIMULTANEOUS EVALUATION OF THE ACUTE EFFECTS OF CHEMICALS ON SEVEN-AQUATIC SPECIES

WILLIAM S. EWELL,* JOSEPH W. GORSUCH, ROBERT O. KRINGLE, KENNETH A. ROBILLARD and RICHARD C. SPIEGEL
Health and Environment Laboratories, Eastman Kodak Company, Rochester, New York 14650

(*Received* 4 *November* 1985; *Accepted* 20 *March* 1986)

Abstract—A cost-effective testing procedure is described that measures the acute aquatic effects of a single test chemical on seven aquatic species simultaneously: *Asellus intermedius* (pillbug), *Daphnia magna* (water flea), *Dugesia tigrina* (flatworm), *Gammarus fasciatus* (sideswimmer), *Helisoma trivolvis* (snail), *Lumbriculus variegatus* (segmented worm) and *Pimephales promelas* (fathead minnow). These species were chosen because of their ecological importance, diversity and amenability to laboratory culturing. Twenty-seven commercial inorganic and organic chemicals were tested using the simultaneous exposure procedure. The 96-h LC50 values were derived for each species and the results compared with literature values. The reproducibility of the results achieved using this testing procedure was shown to be very good. The results compare favorably with 96-h LC50 values from single-species tests.

The susceptibility of test animals as a function of species and chemical compound was evaluated. Water fleas were found to be as susceptible as, or more so than, the other aquatic organisms for all compounds tested. The relative sensitivities of the other test species were found to be highly chemical dependent.

Keywords—Multispecies testing Aquatic toxicity Fathead minnow Aquatic invertebrates Organic and inorganic chemicals

INTRODUCTION

Investigations into multispecies testing have n prompted by a desire to provide a better ns of determining the range of sensitivities of atic animals for use in hazard and risk assessit [1]. The majority of reported toxicity investions describing the impact of chemicals and nology on aquatic environments have been ormed using single-species test methods, usu- with freshwater fish [2–4]. Macroinvertees, primarily from the phylum Arthropoda [5], pose most of the remaining data.

Currently, the range of effects of a chemical on iple aquatic species is obtained in one of two : (a) test one species and apply an application r that estimates relative species sensitivities or erform several single-species tests. The first oach, while having the advantage of involv- single test, has the disadvantage of not estabig the accuracy of the application factor, which may be underprotective or overprotective. In the second approach, a more accurate measurement of community effect is achieved, but greater labor to perform several tests is needed. A cost-effective, reproducible method of assessing the impact of a chemical on several aquatic organisms simultaneously would be of significant advantage in environmental effects testing.

In our laboratory we have developed a test method that achieves the advantages of both of the above approaches, i.e., data is obtained on several species while performing one test. The intent in developing the multispecies test was to expand upon established procedures using accepted methodologies [6–8]. The method evaluates the potential of a substance to cause acute aquatic effects in seven juvenile aquatic species simultaneously. The information from this primary sensitivity test may be useful in predicting the likelihood of an effect should a test chemical enter the freshwater environment.

Immature stages of organisms are used because they are considered more sensitive than

o whom correspondence may be addressed.

adults [9–11]. Species for this acute aquatic effects bioassay were chosen based on their diversity, amenability to laboratory culture and ecological importance [12–19].

An evaluation of the multispecies method using a series of commercially important, large-volume inorganic and organic chemicals was performed. The 96-h LC50 values were derived for each species using the statistical procedures of Stephan [20]. These results were then compared with values in the literature. The aquatic toxicity literature for this series of commercial chemicals predominantly contains information from single-species tests, primarily those with water fleas and fish. The precision of this multispecies method was determined by repeating the testing on one-third of the chemicals after a 6-month interval. The diversity of effects among chemicals and species was the primary requirement in selecting specific chemicals for retesting. One organic chemical and one inorganic chemical were tested on each of the seven aquatic species using conventional single-species test methods [6–8] and the results of the different procedures were compared. Finally, the multispecies method provides an excellent opportunity to compare relative species sensitivities because exposures to each chemical were conducted under identical conditions.

EXPERIMENTAL DESIGN

Test vessel and diluent water

The static multispecies bioassays were performed in seamless glass, 30.5-cm cuboidal, Pyrex chromatography jars to which 20 liters of test solution was added. Water quality was routinely monitored to characterize the diluent water and ensure its suitability [7]. Activated carbon–filtered, dechlorinated and tempered industrial service water from Lake Ontario was used in all tests. The chemical characteristics of the diluent water are presented in Table 1.

Test solutions

The chemicals used in the tests were reagent-grade. Based on a recommended expected environmental concentration cutoff level [21], the maximum concentration used in these bioassays was 100 mg/L. Chemicals known to be readily soluble at 100 mg/L were added directly to the diluent water in each aquarium in the appropriate amounts to give nominal concentrations of 100, 10, 1 and 0.1 mg/L. Test chemical concentrations were not analyzed.

Once the test solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration

Table 1. Chemical characteristics of dilution water (average sample population of 23)

Element	Concn. (mg/L)	Other parameters	Value
Aluminum	0.25	Total dissolved solids	180 mg/L
Arsenic	<0.005	Total hardness ($CaCO_3$)	130 mg/L
Barium	<0.02	Noncarbonate hardness (as $CaCO_3$)	38 mg/L
Cadmium	<0.01	Alkalinity (as $CaCO_3$)	93 mg/L
Calcium	39	Soluble sulfate (SO_4^{-2})	31 mg/L
Chromium	<0.02	Chloride by I.C. (as Cl^-)	26 mg/L
Cobalt	<0.02	Total NH_3 (as N)	0.06 mg/L
Copper	<0.01	Organic nitrogen	0.21 mg/L
Iron	<0.01	NO_3^- (as N)	0.29 mg/L
Lead	<0.05	NO_2^- (as N)	<0.01 mg/L
Lithium	<0.01	Molybdenum-reactive dissolved SiO_2	0.35 mg/L
Magnesium	6.9	Total phosphorus (P)	0.02 mg/L
Manganese	<0.4	Total cyanide (CN^-)	<0.005 mg/L
Mercury	<0.0002	Soluble fluoride (F^-)	0.35 mg/L
Molybdenum	0.24	pH	7.4 pH units
Nickel	0.04	Conductivity	260 μmhos/cm
Potassium	6.7	Total organic carbon	1.8 mg/L
Selenium	<0.002	Total residual Cl_2	6.6 μg/L
Silver	<0.01		
Sodium	10		
Tin	<0.2		
Zinc	<0.01		

I.C. = ion chromatography.

nd the control. When the starting pH of the test olution fell outside the extremes of 6.5 to 8.5, the H was adjusted to 7.0 by the addition of 10% /v) NaOH or 10% (v/v) H_2SO_4.

st organisms

The method simultaneously exposes seven spe- s from five phyla (Table 2). All test organisms re acclimated to the control diluent water in the eeding/rearing tanks. Food was withheld for the h preceding start of the test. Juveniles of each cies, as uniform in size as possible, were col- ted from the colonies. Ten juvenile organisms each species were routinely exposed to the test mical in each treatment. Stratified randomiza- n was accomplished by proportioning out no re than 20% of any one species into an aquar- at any one time. Biological loading was kept ow 0.5 gram wet weight per liter of test solu- . The average wet weight for a randomly cho- set of minnows was determined at the start of test. The other six species were not weighed. The minnows and snails were placed in the test els. The remaining five species were segregated elded stainless steel, 55-mesh wirecloth baskets cm in diameter × 7.5 cm in depth). Each bas- was suspended from a 1-rpm motor-driven hanism (Fig. 1) that raised and lowered the ets in the water column. A stainless steel l, slotted every 0.5 cm, facilitated the position- f the baskets so that the submerged volumes ged from one-third to two-thirds during each . The baskets were spaced around the test ves- m so that they did not interfere with each . One-half of the volume of the submerged t was exchanged with the main tank volume minute.

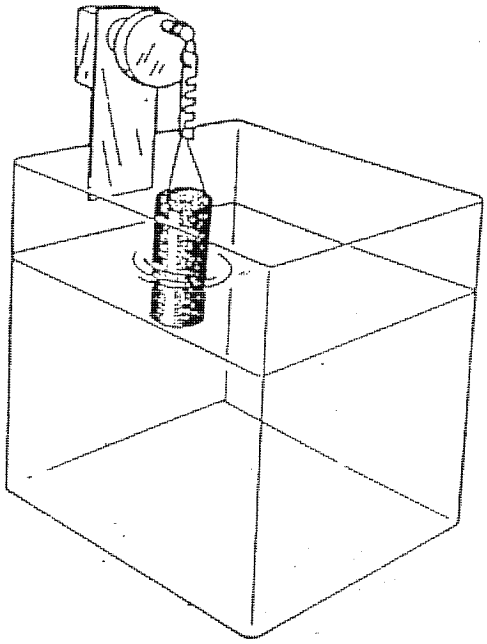

Fig. 1. Containment basket suspended from 1 rpm motor.

cal/chemical parameters

terminations of the temperature, dissolved n and pH of each test solution were made in conjunction with the daily biological observations. The test temperature target was 20 ± 1°C. If the dissolved oxygen concentration in a test chamber fell below 40% of the starting level in a test, the test was repeated with 0.05 L/min glass-sparger aeration. All tests were conducted within the extremes of 6.5 to 8.5 pH units. The photoperiod duration was 16 h of light. The air–water interface of each tank received approximately 50 ft-c of cool-white fluorescent light.

Biological parameters

Biological observations were made daily. Survival, condition and behavorial information were

Table 2. Routinely tested species

on name	Genus and species	Phylum	Approximate size or age
lea	*Asellus intermedius*	Arthropoda	0.012 g
m	*Daphnia magna*	Arthropoda	First and second larval instar
mmer	*Dugesia tigrina*	Platyhelminthes	0.006 g
	Gammarus fasciatus	Arthropoda	0.007 g
ted worm	*Helisoma trivolvis*	Mollusca	0.180 g
minnow	*Lumbriculus variegatus*	Annelida	0.006 g
	Pimephales promelas	Chordata	0.2–0.5 g

Table 3A. 96-h LC50 values for seven freshwater species compared with literature LC50 values for organic compounds (mg/L)

	Water flea	Flatworm	Snail	Minnow	Sideswimmer	Pillbug	Segmented worm
Acetone $(CH_3)_2CO$	>100 (>100)[a]	>100	>100	>100 (>100)[b]	>100	>100	>100
Acetonitrile CH_3CH	>100 (>100)[c]	>100	>100	>100 (>100)[d]	>100	>100	>100
Allyl alcohol CH_2:$CHCH_2OH$	0.25	1.0	4.8	0.32	21	1.0	0.32
Aniline $C_6H_5NH_2$	0.21 (≥0.2-0.65)[e]	31.6	100	32 (65)[b]	>100	>100	>100
Benzoic acid sodium salt C_6H_5COONa	>100 (>100)[f]	>100	>100	>100	>100	>100	>100
Diethanolamine $NH(CH_2CH_2OH)_2$	>100 (1.0-55)[g]	>100	>100	>100 (>100)[h]	>100	>100	>100
Ethyl alcohol C_2H_5OH	>100 (>100)[i]	>100	>100	>100	>100	>100	>100
Ethylenediamine dihydrochloride $NH_2CH_2CH_2NH_2 \cdot 2HCl$	2.5 (0.88-16)[j]	>100	32	>100 (>100)[k]	>100	>100	>100
Methyl alcohol CH_3OH	>100 (>100)[l]	>100	>100	>100	>100	>100	>100
Pentachlorophenol C_6Cl_5OH	0.32 (0.48-0.80)[m]	0.75	0.40	0.32 (0.21-0.6)[n]	0.21	3.2	3.2
Phenol C_6H_5OH	4.0 (12-31)[o]	32	>100	32 (29-32)[p]	21	25	>100
Resorcinol C_6H_4-1,3-$(OH)_2$	0.25	>100	>100	40 (53.4-60.0)[q]	>100	>100	>100
Sodium ferric EDTA $C_{10}H_{12}FeN_2NaO_8$	32 (>100)[h]	>100	>100	>100	100 (>100)[h]	>100	>100
Thymol 5-$CH_3C_6H_3$-2-$CH(CH_3)_2$-1-OH	3.2	5.9	32	3.2	3.2	17	3.2
Triethyl phosphate $(C_2H_5O)_3PO$	>100	>100	>100	>100	>100	>100	>100

LC50 values for this study are based on nominal concentrations. Values in parentheses are limits of literature values.

References: [a]23, 24, 25, 26; [b]26; [c]25; [d]27, 28; [e]24, 25, 26, 29, 30; [f]23, 25, 31; [g]32, 33; [h]33; [i]23, 25, 34; [j]25, 33; [k]33, 35, 36; [l]25, 34; [m]24, 26, 32, 37; [n]26, 38, 39, 40, 41, 42; [o]25, 32; [p]38, 42, 43, 44; [q]35, 45.

Table 3B. 96-h LC50 values for seven freshwater species compared with literature LC50 values for inorganic compounds (mg/L)

	Water flea	Flatworm	Snail	Minnow	Sideswimmer	Pillbug	Segmented worm
Ammonium sulfate $(NH_4)_2SO_4$	>100 (>100)*	>100	>100	>100	>100	>100	>100
Beryllium chloride $BeCl_2$	0.40 (1.0)*[b]	>100	>100	>100 (15)*[c]	5.9	>100	>100
Cobalt chloride $CoCl_2$	3.2 (5)*[d]	25	>100	48	>100	>100	>100
Cupric sulfate $CuSO_4$	0.05 (0.01–0.08)*[e]	3.2	0.32	2.1 (0.55–1.4)*[f]	0.39 (0.19–0.55)*[g]	32	0.32 (0.15)*[h]
Nickel chloride $NiCl_2$	3.2 (<0.32–16)*[i]	32	3.2	40 (20–44.5)*[j]	>100	75	32
Potassium bromide KBr	>100 (>100)[k]	>100	>100	>100 (>100)[l]	>100	>100	>100
Potassium dichromate $K_2Cr_2O_7$	0.21 (0.05–1.4)*[m]	40	32	>100 (52–>100)*[n]	0.32	15	32 (13.3)*[h]
Potassium ferrocyanide $K_4Fe(CN)_6$	32 (0.18)[o]	>100	>100	>100 (>100)[o]	>100	>100	>100
Sodium cyanide NaCN	0.17 (0.16–1.8)[p]	4.0	>100	0.32 (0.13–0.35)[q]	1.7	3.2	21
Sodium hypochlorite NaClO (5.25%)	2.1	32	59	10 (5.9)[r]	4.0	32	3.2
Sodium nitrite $NaNO_2$	48	>100	59	>100	32	>100	>100
Zinc sulfate $ZnSO_4$	0.75 (0.1–14)*[s]	32	32	17 (0.87–9.2)*[t]	10	32 (56)*[h]	32 (6.3)*[h]

LC50 values for this study are based on nominal concentrations. Values in parentheses are limits of literature values. Asterisks indicate literature values in mg/L of metal ion.
References: [a]23; [b]25, 32; [c]46; [d]47; [e]5, 25; [f]5, 46; [g]5, 48; [h]5; [i]25, 47, 49; [j]46, 50, 51; [k]52; [l]53; [m]5, 25, 33, 52; [n]33, 36, 41; [o]33; [p]25, 54, 55; [q]28, 56, 57, 58; [r]35; [s]5, 56, 59.

recorded. Dead organisms were removed when observed. A test organism was considered dead if it appeared motionless and exhibited no response to gentle prodding. If more than one-half of the population of a species exposed in any treatment was determined to be dead, additional aquaria containing lower concentrations of test solution were set up. All seven species were exposed to each dose level. At any time during the test when all 10 organisms of a species were considered dead, these biological parameters were determined and recorded.

All species in these tests were exposed for the same time period, 96 h. As in any bioassay that determines a dose response, the LC50 value can be achieved at any time during the exposure. It is recognized by the authors that some invertebrates usually are exposed for only 48 h.

Equivalency of multispecies and single-species test data

To evaluate the equivalency of 96-h LC50 values obtained from this multispecies test with corresponding 96-h LC50 values from single-species tests, duplicate trials were conducted simultaneously with each procedure. A multispecies test was performed together with seven single-species tests, one for each type of aquatic animal. Two chemicals were tested in this manner: thymol and zinc sulfate (heptahydrate).

Data analysis

Estimation of LC50 values. The LC50 values were estimated by an interpolation method using a computer program written for aquatic toxicity studies [20]. This linear interpolation uses the logarithm transformation of the concentration and the angle transformation of the percent dead between the two doses that bracket 50% response (i.e., proportion killed). The moving average and probit methods could not be used because the required minimum of two partial mortalities was rarely obtained.

Repeatability (precision) of test procedure. For each of the nine compounds tested twice, a single degree-of-freedom estimate of the variance associated with the log LC50 was computed for each species. These single degree-of-freedom variances were then averaged over compounds and species to obtain an estimate of the variance of the toxicity test procedure within this laboratory.

Species sensitivity comparison. To compare the sensitivities of the seven species, we used the original set of log LC50 values (i.e., without the repeat test data). Pairwise species comparisons of log LC50 values, by compound, were made with Fisher's least significant difference procedure [22], using the standard deviation from the repeatability analysis.

RESULTS

Estimation of LC50 values

The LC50 values are given in Table 3A and B. Values are expressed in milligrams per liter, as the chemical without any water of hydration. Some LC50 values could not be estimated because the proportion killed at the highest dose level was less than 50%. These values are expressed as greater than 100 mg/L.

Comparison with literature values

Results from the multispecies tests were compared with extremes of LC50 values reported in the literature for the same chemicals and species. The literature values referenced in Table 3A and B for certain invertebrates may be from bioassays of a duration less than 96 h. The extremes of the literature values from conventional single-species tests are given in parentheses in Table 3A and B.

Repeatability (precision) of test procedure

The LC50 values for the nine compounds that were tested twice are given in Table 4. For 34 of the 63 pairs, both tests provided a log LC50, so that 34 single degree-of-freedom estimates of the variance associated with predicting the log LC50 were obtained. The pair of log LC50 values for pentachlorophenol with pillbugs was omitted as an outlier. The remaining 33 pairs of log LC50 values yield an average variance (S^2) of 0.04. Thus, the estimated standard deviation (S) associated with a predicted log LC50 is 0.20. Based on these data, a value of 0.20 can be interpreted as an "average" standard deviation for this multispecies test. Ninety-five percent confidence limits (C.L.) for an estimated log LC50 value are given by

$$95\%\ \text{C.L.} = \log \text{LC50} \pm (1.96 \times 0.20)$$

Thus, the maximum uncertainty associated with the log LC50 is $\pm 1.96\ (0.20) = \pm 0.39$, with 95% confidence.

Multispecies versus single-species test comparison

The LC50 values for replicate tests with thymol and zinc sulfate (heptahydrate) are given in Table 5A and B. Excellent agreement between the two

Table 4. Repeatability (precision) of test procedure 96-h LC50 values (mg/L)

	Test No.	Water flea	Flatworm	Snail	Minnow	Sideswimmer	Pillbug	Segmented worm
Allyl alcohol	1	0.25	1.0	4.8	0.32	21	1.0	0.32
CH_2:$CHCH_2OH$	2	0.40	4.0	4.8	0.32	4.9	0.32	0.10
Diethanolamine	1	100	100	100	100	100	100	100
$NH(CH_2CH_2OH)_2$	2	100	100	100	100	100	100	100
Nickel chloride	1	3.2	32	3.2	40	100	75	32
$NiCl_2$	2	3.2	32	3.2	100	100	100	48
Pentachlorophenol	1	0.32	0.75	0.40	0.32	0.21	3.2	3.2
C_6Cl_5OH	2	0.32	0.32	0.75	0.32	0.48	32	3.2
Phenol	1	4.0	32	100	32	21	25	100
C_6H_5OH	2	4.0	59	100	40	25	100	100
Potassium ferrocyanide	1	32	100	100	100	100	100	100
$K_4Fe(CN)_6$	2	75	100	100	100	100	100	100
Sodium ferric EDTA	1	32	100	100	100	100	100	100
$C_{10}H_{12}FeN_2NaO_8$	2	13	100	100	100	100	100	100
Sodium nitrite	1	48	100	59	100	100	100	100
$NaNO_2$	2	41	100	100	100	32	100	100
Thymol	1	3.2	5.9	32	3.2	32	100	100
5-$CH_3C_6H_3$-2-$CH(CH_3)_2$-1-OH	2	1.7	4.0	32	3.2	3.2	17	3.2
						3.2	25	4.8

LC50 values for this study are based on nominal concentrations.

Table 5A. Comparison of multispecies test and single-species test for thymol (mg/L)

Test	Trial	Dm	Dt	Ht	Pp	Gf	Ai	Lv
Single	1	3.2	3.6	32	4.2	3.2	28	10*
Multi	1	3.2	3.2	32	3.2	3.2	24	3.2
Single	2	3.2	3.2	32	3.2	3.2	28	3.2
Multi	2	3.2	3.2	32	3.2	3.2	19	3.2

Dm, water flea; Dt, flatworm; Ht, snail; Pp, fathead minnow; Gf, sideswimmer; Ai, pillbug; Lv, segmented worm.

*Five of ten segmented worms at 10 ppm were fragmented but still moving at 96 h. Since the endpoint was mortality, the LC50 point estimate was calculated to 10 mg/L.

Table 5B. Comparison of multispecies test and single-species test for zinc sulfate (heptahydrate; mg/L)

Test	Trial	Dm	Dt	Ht	Pp	Gf	Ai	Lv
Single	1	0.8	32	32	32	24	4.7	32
Multi	1	0.7	32	32	22	6.7	5.5	22
Single	2	1.2	32	32	15	4.5	>100	32
Multi	2	2.2	32	32	13	25	>100	32

Dm, water flea; Dt, flatworm; Ht, snail; Pp, fathead minnow; Gf, sideswimmer; Ai, pillbug; Lv, segmented worm.

test procedures was observed for all species exposed to thymol and six species exposed to zinc sulfate. Although the LC50 values for sideswimmers were reversed from trial 1 to trial 2, the extremes remained the same.

Species sensitivity comparison

Species sensitivity comparisons, by compound, were conducted using Fisher's least significant difference procedure. With this procedure, any two log LC50 values are statistically different ($\alpha = 0.01$) if they differ by at least $(2.576)(0.20^2 + 0.20^2)^{1/2} = 0.73$. This procedure was applied to determine the most sensitive species for each compound. Nine compounds were omitted because none of the seven LC50 values were estimable.

Although some LC50 values could be computed for potassium ferrocyanide and sodium nitrite, these LC50 values were not statistically distinguishable among the other species and were also omitted. The results for the remaining 16 compounds are given in Table 6A and are summarized in Table 6B.

DISCUSSION

We demonstrated that this multispecies testing procedure is a reliable, precise and accurate method for establishing the magnitude of acute aquatic effects of a chemical on representatives of

Table 6A. Most sensitive by compound on the basis of Fisher's least significant difference multiple comparison criterion

Compound	Most sensitive species
Allyl alcohol	Dm = Dt = Pp = Ai = Lv
Aniline	Dm
Beryllium chloride	Dm
Cobalt chloride	Dm
Cupric sulfate	Dm
Ethylenediamine dihydrochloride	Dm
Nickel chloride	Dm = Ht
Pentachlorophenol	Dm = Dt = Pp = Gf = Ht
Phenol	Dm = Gf
Potassium dichromate	Dm = Gf
Resorcinol	Dm
Sodium cyanide	Dm = Pp
Sodium ferric EDTA	Dm
Sodium hypochlorite	Dm = Gf = Pp = Lv
Thymol	Dm = Dt = Pp = Gf = Ai = Lv
Zinc sulfate	Dm

Dm, water flea; Dt, flatworm; Ht, snail; Pp, fathead minnow; Gf, sideswimmer; Ai, pillbug; Lv, segmented worm.

Table 6B. Percentage of compounds for which each species is the single most sensitive or equally most sensitive

	Dm	Dt	Ht	Pp	Gf	Ai	Lv
Single most sensitive	50	0	0	0	0	0	0
Equally most sensitive	100*	19	13	31	31	13	19

Dm, water flea; Dt, flatworm; Ht, snail; Pp, fathead minnow; Gf, sideswimmer; Ai, pillbug; Lv, segmented worm.

*Includes single most sensitive determinations.

several phyla in a minimum of time and with minimal use of laboratory resources. The majority of the LC50 values estimated using the multispecies method fell within the extremes of established literature values. The repeatability of the tests is such that, for the present set of chemicals, the 95% confidence intervals equal the log LC50 ± 0.39.

The comparison of species sensitivities using Fisher's least significant difference procedure indicated that even though water fleas were always ranked among the most sensitive species, they were joined half the time by other species. This apparent increased sensitivity of water fleas may be due to the greater duration of a 96-h test. It should be noted that 5 of 21 water flea 96-h LC50 values were below the lower extreme of the literature values. However, 2 of 21 water flea 96-h LC50 values were higher than the upper extreme of the literature values. The remaining 14 water flea 96-h LC50 values were within the extremes of the literature values.

CONCLUSIONS

This method for simultaneously exposing several species in an aquatic bioassay has proven to be reliable and repeatable. It requires relatively little additional time and facilities beyond those necessary for currently established testing programs. Water fleas were found to be as susceptible as, or more so than, the other aquatic organisms for all compounds tested. The relative sensitivities of the other six test species were found to be chemical dependent. This multispecies method provides data equivalent to those obtained in conventional single-species tests.

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Photochemical Transformation in Aqueous Solution and Possible Environmental Fate of Ethylenediaminetetraacetic Acid (EDTA)[1]

RUDOLF FRANK AND HERMANN RAU

Universität Hohenheim, Institut für Chemie 130, Fachgebiet Physikalische Chemie, 7000 Stuttgart 70, Federal Republic of Germany

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INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) is used in a large variety of products, e.g., laundry detergents, cosmetics, photochemicals, and pharmaceuticals, and in industries, e.g., textiles and galvanic and paper manufacturing. The content of EDTA in laundry detergents varies between 0.1 and 0.5%. For the FRG, therefore, a consumption of 1000 to 5000 *t/a* can be estimated for this purpose alone. Laundry detergents account for an estimated 20 to 30% of the total EDTA consumption which thus may amount up to 25,000 *t/a*.

Because EDTA is not volatile, it is released mainly with waste water streams. In waste water treatment plants EDTA is neither transformed by microorganisms nor adsorbed to the sewage sludge (Gardiner, 1976; Means *et al.*, 1980). Taking into account the figures mentioned above and the amount of waste water of the FRG, concentrations of 50–500 μg/liter can be estimated for waste waters. Due to dilution, environmental concentrations in German rivers are expected to be lower by a factor of 1–3. Measured concentrations in natural waters were reported to range from 10 to 70 μg/liter, with a median value of 23 μg/liter (Dietz, 1984). These figures are confirmed in reports of the Arbeitsgemeinschaft Rhein-Wasserwerke e. V. (ARW, 1986) and the Eidg. Anstalt für Wasserversorgung Abwasserreinigung und Gewässerschutz (EAWAG, 1987). Comparison of estimated and measured environmental concentrations confirms that most of the EDTA used is released to surface waters without chemical transformation.

Analytical determinations of EDTA do not provide information about the chemical bonding of EDTA in surface waters (Reichert *et al.*, 1980; Linckens and Reichert, 1982). Because it is a strong complexing agent, it seems appropriate to assume that it is present in the form of its metal complexes. Taking into account the pH-dependent effective complexing constants (Kober, 1979; Gmelin, 1987) and the concentrations of metals (including Ca and Mg) in surface waters (Bayerisches Landesamt, 1983; Landesanstalt, 1983), it can be estimated that most of the EDTA should be present in the form of Fe(III) complexes. On the other hand, most of the iron in surface waters is present in the form of colloidal and amorphous iron hydroxides and therefore may not be complexed by EDTA. Calculations for nitrilotriacetic acid (NTA), therefore, lead to the conclusion that most of this compound in surface waters occurs in the form of its copper complexes (NTA, 1984). However, the Fe(III)–EDTA complex is

[1] This work has been presented in part at the XII IUPAC Symposium on Photochemistry in Bologna, July 1988.

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the most stable EDTA complex (pH 7) and EDTA can react with Fe(III) already at the waste water treatment plant. This possibility will be especially enhanced in the future when an increasing number of these plants will be equipped with phosphate eliminating stages.

As mentioned above, for the most part EDTA is not transformed by aerobic microorganisms in waste water treatment plants and transformation by microorganisms in nature is extremely slow also. For the removal of EDTA from surface waters photochemical reactions and reactions with other reactive species such as OH, singlet oxygen, H_2O_2, etc., therefore could be the only sinks.

In earlier publications, ethylenediaminetri-, di-, and monoacetic acid have been identified as photo products (Lockhart and Blakeley, 1975). Published quantum yields for FeEDTA range from 0.003 to 0.35 (Carey, 1973, 1975; Natarajan and Endicott, 1973). In these experiments, the FeEDTA concentrations have been much higher than environmental concentrations. Because these reports hint that the quantum yield depends on pH, excitation wavelength, and concentration it seemed appropriate to study the kinetics of the photoreaction in detail at concentrations similar to environmental concentrations. A further aim of this work is to estimate the possible environmental fate of EDTA and calculate its environmental half-life.

EXPERIMENTAL METHODS

EDTA sodium salt, analytical grade, was purchased from Merck and used without further purification. Water from an ion exchanger was doubly distilled in a quartz apparatus.

The Fe(III) complexes form immediately after mixing of equimolar solutions. Most of the kinetics experiments were performed in 0.1 M $NaClO_4$ solutions but the results of single experiments in sodium acetate and in pure water did not deviate from the results in $NaClO_4$.

For the irradiation with monochromatic light, single lines from a high-pressure mercury lamp (Philips HPK 125) were separated with precision interference filters (Schott UV-PIL Filter). The irradiation intensities were determined using the azobenzene actinometer (Gauglitz 1976; Gauglitz and Hubig, 1981). For uv measurements a spectral photometer (Zeiss DMR 10) was used.

Water samples from the Neckar river were taken from the surface. All samples were clear although the river appeared to be dark. The optical transmission of the samples was measured within approximately 30 min after taking the sample in a spectrophotometer.[2] No sedimentation of dissolved material could be observed visually.

RESULTS AND DISCUSSION

Reactive Species

The uv absorption spectrum of FeEDTA shows a maximum at 258 nm in acidic solution and at 242 nm in basic solution. The titration spectrum is shown in Fig. 1. The isosbestic point at 244 nm reveals that within the tested pH range (2.5–10.5) and the high dilution (ca. 1×10^{-4} M) only two differently protonated forms of the FeEDTA complex can occur. The formation of dimers, which has been

[2] The optical transmission data of the Neckar river are available as ASCII files. If you are interested in these data please send a formatted disc (5.25" MS-DOS) to the authors.

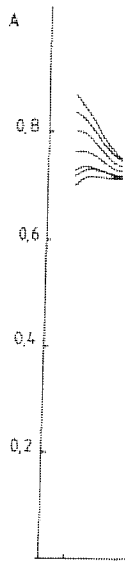

FIG. 1. Titration spectrum of F
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Determination of Photochem

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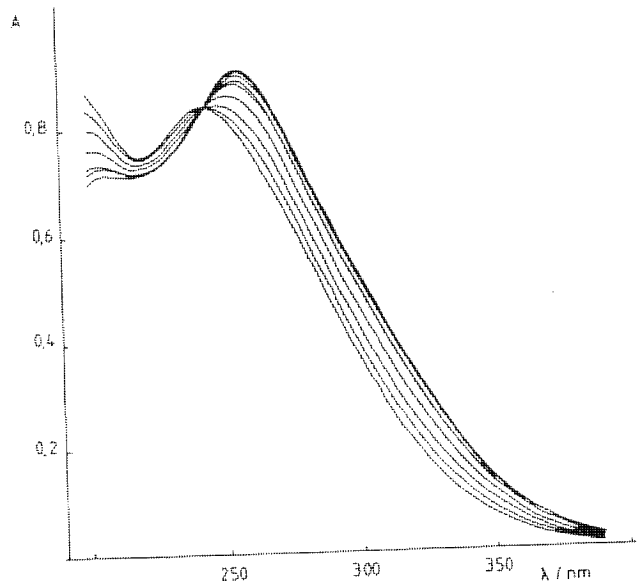

FIG. 1. Titration spectrum of FeEDTA. In acid (pH 2.5) solution the absorption maximum is situated at 258 nm; in basic (pH 10.5) solution the maximum is situated at 242 nm.

observed previously (Schugar *et al.*, 1969) and which can cause concentration-dependent quantum yields, is observed only at higher concentrations. At environmental concentrations, therefore, only photoreactions of the monomeric complexes are to be expected. In FeEDTA the EDTA trianion is active as a pentadentate ligand and the sixth coordination position in the Fe(III) ion is occupied by a water molecule. In basic solutions this water molecule is assumed to lose its proton (Schugar *et al.*, 1969). The pK_A value of the complex is 7.5. In natural surface waters, the neutral as well as the negatively charged complex can appear as the photoreactive species.

The ground state of the FeEDTA complex is a sextett. Hence, d–d absorption bands are not expected. This is confirmed by the location of the first absorption band in the spectrum at 258/242 nm. This absorption is attributed to a ligand to metal charge transfer state (Schugar *et al.*, 1969).

Determination of Photochemical Quantum Yields

Quantum yields have been determined in dilute solutions ranging from 0.04 $\times 10^{-4}$ to 1.7 $\times 10^{-4}$ M at various pH values in oxygen-free (argon-saturated) and air-saturated solutions. The solutions were irradiated with monochromatic light at 313, 366, and 405 nm. A typical reaction spectrum is shown in Fig. 2. Absorption diagrams (Mauser, 1974; Rau, 1989 [appendix]) reveal that FeEDTA is the only light-absorbing substance in the wavelength interval depicted in Fig. 2. Therefore, the quantum yield can be determined using Eq. (1) (Mauser, 1974).

$$\ln A(\lambda, t) = \ln A(\lambda, t_0) - 10^3 I_0 \psi \epsilon'_A \int_{t_0}^{t} \frac{1 - 10^{-A'}}{A'} dt. \qquad (1)$$

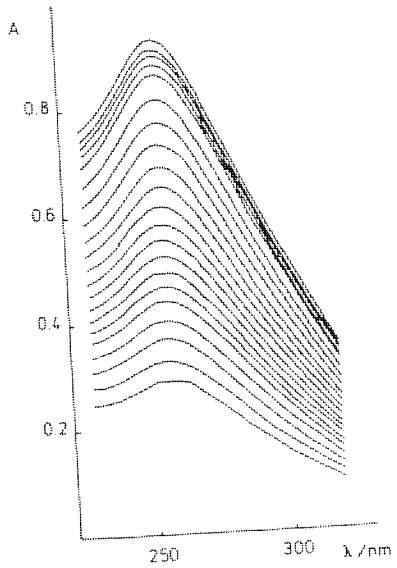

FIG. 2. Reaction spectrum of FeEDTA at pH 4. Irradiation wavelength is 313 nm.

With

$A(\lambda, t)$ = Absorbance of FeEDTA at wavelength λ and time t.

A' = Absorbance of FeEDTA at the irradiation wavelength at time t.

I_0 = Irradiation intensity in mol photons cm^{-2} sec^{-1}.

ψ = Quantum yield of FeEDTA at the irradiation wavelength.

ϵ'_A = Molar, decadic absorption coefficient of FeEDTA at the irradiation wavelength in M^{-1} cm^{-1}.

t = Irradiation time in sec.

Figure 3 shows a typical diagram of ln $A(\lambda, t)$ versus the integral of Eq. (1). The integral is stepwise numerically evaluated from the measured values of A' and t. The quantum

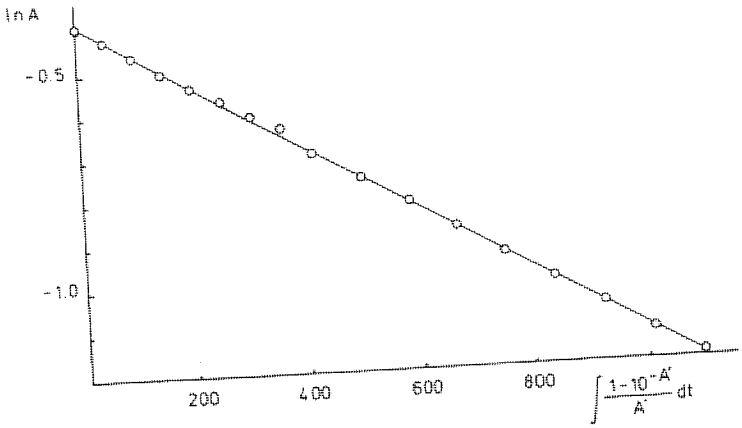

FIG. 3. Plot of ln $A(258, t)$ versus the integral of Eq. (1).

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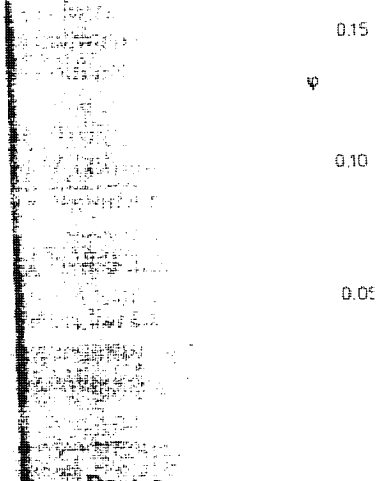

FIG. 4. Quantum yield of FeED
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TABLE I

OPTICAL ABSORPTION COEFFICIENTS OF FeEDTA IN M^{-1} cm^{-1} AT THE IRRADIATION WAVELENGTHS AT VARIOUS pH VALUES

pH	ϵ_{313}	ϵ_{366}	ϵ_{405}
2.5–6.2	4300		
4.0	4300	800	150
6.8	4030		
7.55	3360		
8.0	2950	450	75
8.4	2750		
9.2	2590		
9.5	2590		
10.3	2550		

yield is then determined from the slope of these diagrams and measured values of the irradiation intensity and the optical absorption coefficients. The absorption coefficients used for the calculations are given in Table 1. The quantum yield was determined as a function of pH and irradiation wavelength. The results are given in Figs. 4 and 5.

Due to the various errors which occur in determining the slope, the irradiation intensity, and the absorption coefficients the error bars in these figures are estimated by error propagation. Figure 4 shows the quantum yield as a function of pH when light of 313 nm is used for irradiation. In oxygen-free solution, a clear difference in the quantum yield of the protonated and deprotonated FeEDTA complex can be recognized. The quantum yield for the protonated complex is 0.133 and for the deprotonated complex 0.07. In the presence of oxygen, this difference is less pronounced, which can be due to various reasons. In the FeEDTA complex excitation by light causes an electron transfer from the ligand to the central Fe(III). The primary photoproducts from this molecule can be an Fe(II) ion and an alkyl-radical. Accord-

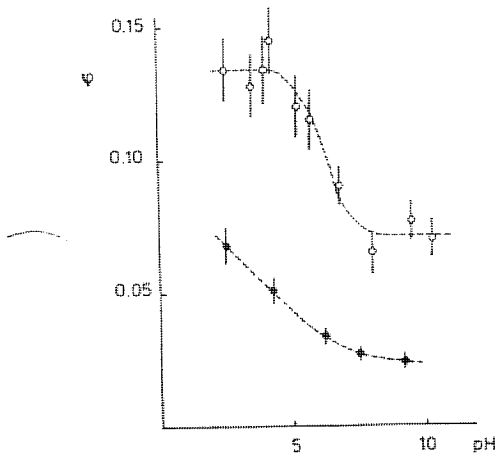

FIG. 4. Quantum yield of FeEDTA versus pH. Irradiation wavelength is 313 nm. Open circles, oxygen-free (argon-saturated) solutions; filled circles, air-saturated solutions.

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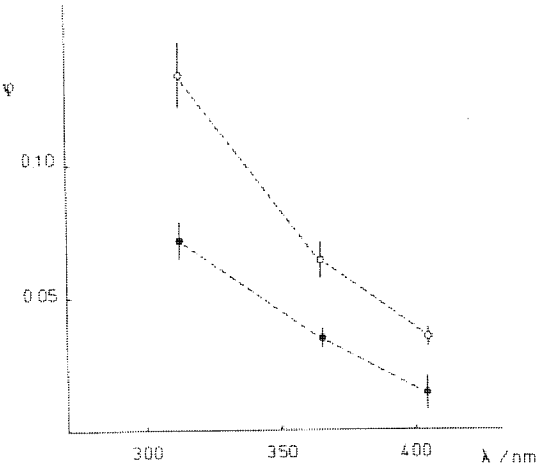

FIG. 5. Quantum yield of FeEDTA versus irradiation wavelength in oxygen-free solutions. Open circles, at pH 4; filled circles, at pH 8.

ing to Carey and Langford (1973, 1975) this radical can be stabilized by reaction with another FeEDTA. The reaction therefore could reach a limiting quantum yield of 2. This stabilization reaction becomes less probable in highly diluted solutions and certainly has no significance in environmental photochemistry. If the reaction proceeds via the suggested path then lower quantum yields should be observed, if there are other molecules in the solution (radical scavengers) with which the primary alkyl-radical can react.

Typical radical scavengers such as ethanol, methanol, and 2-propanol in concentrations up to 0.3 *M* and excess EDTA lower the quantum yield only slightly by approximately 20%. But in the presence of these compounds the photo reaction becomes less reproducible and the resulting quantum yields show a larger scatter. The addition of saccharose does not change the quantum yield.

Oxygen also known as an effective radical scavenger lowers the quantum yield to a much larger extent as can be seen in Fig. 4 and the difference in the reactivity of the protonated and deprotonated complex is less pronounced in the presence of oxygen. Oxygen can change the quantum yield by at least two mechanisms. It can react with the primary alkyl-radical and thereby prevent the reaction of this radical with a further FeEDTA molecule and it can physically quench the excited FeEDTA complex and thereby prevent the primary photo reaction. It can further reoxidize the Fe(II) formed in the primary reaction to Fe(III). Because the Fe(III) complexes with EDTA are much more stable than the Fe(II) complexes, this can be true for ethylenediamine-triacetic acid and the various diacetic acids which are formed by the photoreaction also. At environmental concentrations, these reactions will be of no concern. But in laboratory experiments the presence of oxygen causes the experiments to become less reproducible. Comparison of the effects of the various radical scavengers on the quantum yield shows it is probable that the effect of oxygen is due to the physical quenching of the excited FeEDTA complex as well as to the reaction with the primary alkyl-radical. The importance of this effect with respect to an assessment and the estimation of environmental life times of EDTA will be discussed later.

Figure 5 shows the dependence of the quantum yield on the irradiation wavelength at pH 4 and pH 8. Contrary to organic molecules both complexes exhibit a depen-

dence of the quantum yield on irradiation wavelength with a clear decrease in the quantum yield with increasing irradiation wavelength. In organic molecules, photoreactions normally start from the lowest, clearly isolated singlet or triplet electronic states; this is known as Kasha's rule. In metal complexes, there exist a variety of states which are situated close to one another in energy and which cannot be excited separately. Due to their electronic structure, metal complexes therefore more often exhibit a quantum yield which depends on the irradiation wavelength. However, for FeEDTA another possibility exists to explain this behavior (Natarajan and Endicott, 1973). Natrajan and Endicott suggest that in FeEDTA the photon may be absorbed either by the ligand or by a charge transfer absorption. Whereas the charge transfer state is photoreactive, the ligand excited state is not. According to this suggestion the dependence of the quantum yield should be due to an innermolecular filter effect.

Assessment of the Possible Environmental Fate of EDTA

The transformation of EDTA in natural waters can occur via the photoreaction of the FeEDTA complex. If the quantum yield of this complex, its optical absorption spectrum, the optical transmission of the natural water, and the solar irradiance are known, the rate of this transformation can be calculated. For the performance of these calculations the model of (Frank and Klöpffer, 1989) was used. Quantum yield and absorption spectrum of FeEDTA depend on the pH of the natural water. Oxygen content and the concentration of other substances of the natural water can influence the rate of the photochemical reaction. The differences in rate caused by these factors are of the same order of magnitude as those which are caused by the variations in the environment, mainly due to the variation of the solar irradiance. Taking this into account and trying to get conservative values, for the performance of estimations, it seems to be appropriate to take optical absorption coefficients which belong to the FeEDTA spectrum at pH 7, quantum yields which belong to the same pH value, and an oxygen content which is near the air-saturated value. For the German river Neckar, the authors collected a relatively large number of optical transmission spectra over a relatively long time period. These transmission data were used for the performance of calculations. Different results for the same months reveal the variation of the environmental half-life which is due to the changes in the optical transmission of the river. The results of the calculations are compiled in Table 2.

For all calculations the starting concentration of FeEDTA was set at 8×10^{-6} *M*. The depth of the natural water was set at 2 m and the flow rate to 0.5 $msec^{-1}$. The half-lives in Table 2 are given for averaged maximum, mean, and minimum solar irradiance in central Europe. The flow distance in Table 2 is the mean distance from the emission source within which the starting concentration decreases by a factor of 2. The results compiled in Table 2 show that FeEDTA can be transformed in rivers in central Europe within relatively short times by photochemical reactions during the summer season. In winter, transformation times become so long that most of the FeEDTA will reach the sea. Due to the higher optical transmission of the seawater, photochemical transformation rates in these waters seem to be higher than in rivers. However, the much higher depth of the sea is not taken into account in the calculation given in Table 2. Comparison of calculated transformation rates and measured environmental concentrations in rivers is not yet possible to a large extent, because there are only a limited number of data sets available. The still limited published data of EDTA concentrations (EAWAG, 1987; ARW, 1986), which extend over a longer

TABLE 2

ESTIMATED HALF-LIFE OF FeEDTA IN THE GERMAN RIVER NECKAR IF NOT STATED OTHERWISE

Month, year	Half-life/hr			Flow distance/km
	Min	Mean	Max	
January 1988	240	480	1600	860
1989	47	98	410	180
February 1988	22	46	190	82
March 1989	8	16	70	29
April 1988	7	12	46	21
May 1988	6	10	38	17
June 1988	9	13	51	23
July 1988	7	10	34	19
August 1987	4	5	18	10
1987	4	6	20	11
1988	6	10	31	17
September 1987	14	24	87	44
1988	12	19	70	35
October 1987	25	46	200	83
1988	18	33	150	60
November 1988	39	87	400	160
December 1988	84	180	770	320
Kieler Förde March 1985	3	6	27	

Note. For details see text.

time period, seem to reveal that higher concentrations occur during the winter season; this seems to be due not merely to the lower amount of water in the rivers during winter. The reason for the lower EDTA concentrations observed in summer, therefore, could be photochemical transformation.

Other abiotic transformation processes for FeEDTA could be reactions with OH radicals and singlet oxygen ($^1O_2$). For the reaction with OH radicals, rate coefficients between 4.8×10^8 and 15×10^8 M^{-1} sec^{-1} have been reported (Farhataziz and Ross, 1977; Lati and Meyerstein, 1978). The steady-state concentration of OH radicals in natural waters can reach values of 10^{-16} to 10^{-17} M (Haag and Hoigné, 1985). That means that abiotic reactions with OH radicals could become important if the quantum yield of FeEDTA were as low as 10^{-5}. This estimate is valid for FeEDTA, which has relatively low absorption coefficients in the solar spectrum. For other substances with higher absorption coefficients, this threshold would be even lower.

Rate coefficients for FeEDTA with singlet oxygen have not been published. However, a limiting value for rate coefficients of singlet oxygen with molecules containing electron-rich double bonds seems to be 10^9 M^{-1} sec^{-1} (Wilkinson and Brummer, 1981); for other molecules this value is still lower. The concentration of singlet oxygen in natural surface waters is ca. 10^{-14} M (Scully and Hoigne, 1987). Reactions with singlet oxygen, therefore, will not become important as long as the quantum yield is higher than approximately 10^{-4}; for substances with absorption coefficients in the solar spectrum higher than those of FeEDTA, this limiting value again would be even lower.

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CONCLUSIONS

When EDTA is released with waste water streams, removal will not occur in the waste water treatment plants. In natural waters, EDTA in the form of its differently protonated Fe(III) complexes can be transformed by photochemical reactions. In central Europe, these reactions can be relatively effective in summer but during winter most of the EDTA will reach the sea. Other abiotic processes are not likely to contribute to the degradation of EDTA.

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Review

Safety Assessment of Iron EDTA [Sodium Iron (Fe^{3+}) Ethylenediaminetetraacetic Acid]: Summary of Toxicological, Fortification and Exposure Data

J. HEIMBACH[1]*, S. RIETH[1], F. MOHAMEDSHAH[1], R. SLESINSKI[1], P. SAMUEL-FERNANDO[2], T. SHEEHAN[2], R. DICKMANN[2] and J. BORZELLECA[3]

[1]ENVIRON Corporation, 4350 N. Fairfax Drive, Arlington, VA 22203, [2]Kellogg Company, 235 Porter St, Battle Creek, MI 49016-3423 and [3]ToxPro, 8718 September Drive, Richmond, VA 23229-7319, USA

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Abstract—Iron EDTA [sodium iron (Fe^{3+}) ethylenediaminetetraacetic acid (EDTA)], shown to have a significant beneficial effect on iron status by increasing iron bioavailability in human diets, has been proposed for use as a fortificant in certain grain-based-products including breakfast cereals and cereal bars. This paper presents an assessment of the safety of iron EDTA for its intended uses in these products. Iron EDTA, like other EDTA–metal complexes, dissociates in the gastrointestinal tract to form iron, which is bioavailable, and an EDTA salt; absorption of the metal ion and EDTA are independent. Because of this dissociation, consideration of information on EDTA compounds other than iron EDTA is relevant to this safety assessment. EDTA compounds are poorly absorbed in the gastrointestinal tract and do not undergo significant metabolic conversion. They have a low degree of acute oral toxicity. EDTA compounds are not reproductive or developmental toxicants when fed with a nutrient-sufficient diet or minimal diets supplemented with zinc. In chronic toxicity studies, diets containing as much as 5% EDTA were without adverse effects. EDTA compounds were not carcinogenic in experimental animal bioassays and are not directly genotoxic. This lack of significant toxicity is consistent with a history of safe use of other EDTA compounds ($CaNa_2EDTA$ and Na_2EDTA) approved by the FDA for use as direct food additives. An upper-bound estimated daily intake (EDI) of EDTA from iron EDTA (1.15 mg/kg bw/day for the US population) is less than half the acceptable daily intake (ADI) for EDTA of 2.5 mg/kg bw/day established by JECFA. The data collected and published over the past 20 to 30 years demonstrate that iron EDTA is safe and effective for iron fortification of food products and meets the standard of "reasonable certainty of no harm". Based on the published record, iron EDTA may be regarded as generally recognized as safe (GRAS) for the intended food uses and maximum use levels. © *2000 Elsevier Science Ltd. All rights reserved*

Keywords: iron; EDTA; fortification; nutrient; GRAS.

Abbreviations: ADI = acceptable daily intake; bw = body weight; DV = daily value; EDI = estimated daily intake; GRAS = generally recognized as safe; PVC = packed red cell volume; RTE = ready-to-eat.

Introduction

Iron deficiency anaemia is a serious health condition that results from insufficient intake and/or poor absorption of iron from the diet. The FDA has listed a number of iron compounds as generally recognized as safe (GRAS) with no restrictions other than good manufacturing practices (21 CFR 172). The FDA has affirmed as GRAS the use of several iron compounds both as nutrient supplements and as food additives and supplements in infant formulas (21 CFR 184). Generally, ferric (Fe^{3+}) iron in food and supplements is poorly absorbed by humans because it is precipitated from solution at a pH above 3.5. Attempts to increase

*Corresponding author. Tel: (703) 516-2362; Fax: (703) 516-2390; e-mail: jheimbach@environcorp.com.

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the bioavailability of iron from iron supplements have led to the use of various chelating agents (INACG, 1993). One such agent, iron EDTA (ethylenediaminetetraacetic acid), has been proposed for use as a fortificant in certain cereal-based products.

This paper presents an assessment of the safety of iron EDTA for its intended uses in grain-based products. Included in this safety assessment is a brief overview of EDTA chemistry, the history of food use of EDTA compounds, evidence for the nutritional efficacy and increased bioavailability of iron EDTA, a review of the toxicity of iron and EDTA chelates, and estimates of daily intake projected to result from the intended uses. Iron EDTA, like other EDTA–metal complexes, dissociates in the gastrointestinal tract to a bioavailable form of iron and an EDTA salt; absorption of the metal ion and EDTA are independent. Because of this dissociation, consideration of information on various EDTA compounds in addition to iron EDTA is relevant to this safety assessment. A critical evaluation of this information should result in conclusions regarding its safety under the standard of "reasonable certainty of no harm."

Chemical properties of iron EDTA and other EDTA–metal complexes

Iron EDTA (CAS No. 15708-41-5), also referred to as ferric edetate, ferric sodium edetate, sodium feredetate, edetic acid sodium iron salt, and sodium iron EDTA (JECFA, 1993a), is shown in Fig. 1.

EDTA is a hexadentate chelator capable of binding stoichiometrically with virtually every metal in the periodic table (Chaberek and Martell, 1959) via four carboxylate and two tertiary amine groups. A neutral or anionic metal chelate is formed with divalent or trivalent metal ions. The various EDTA–metal complexes formed depend on their respective affinity constants, the pH of the environment in which they are formed, and the concentration of the competing metals as well as other competing ligands (Hart, 1984; JECFA, 1974; Martell, 1960; Plumb *et al.*, 1950; Wade and Burnum, 1955; West and Sykes, 1960). The stability constants for different EDTA–metal complexes vary considerably and metals forming a stronger complex with EDTA displace the weaker complexes. Among the nutritionally important trace metals, ferric (Fe^{3+}) iron has the highest stability constant followed by copper, zinc, ferrous iron, calcium, magnesium and sodium. Calcium EDTA has been used for the complexing and excretion of lead in individuals with lead poisoning (Klaassen, 1996).

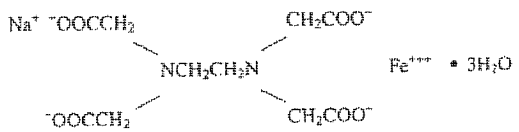

Fig. 1. Sodium iron ethylenediaminetetraacetic acid (NaFeEDTA).

In the gastrointestinal tract, metal ions on the EDTA–metal complex are freely exchanged. Accordingly, the various EDTA salt forms would be expected to have essentially similar properties in the body regardless of the EDTA–metal complex to which exposure occurred. Therefore, available information on various EDTA–metal complexes are considered in this assessment as relevant to a determination of the safety of iron EDTA.

History of food use of EDTA

In the United States, several EDTA salts have a history of safe food use. As direct food additives, they are approved for use (21 CFR 172) as preservatives, processing aids and colour stabilizers in a wide variety of foods, serving as chelating agents to bind trace metal ions that may produce undesirable effects on the colour, clarity, and stability of foods. $CaNa_2EDTA$ is approved for use in foods such as canned carbonated soft drinks, canned cooked vegetables, potato salad, frozen white potatoes, mayonnaise, salad dressings, margarine, sandwich spreads and canned cooked shellfish at concentrations ranging from 25 to 800 ppm. Na_2EDTA is approved for use in a similar variety of food products and in aqueous multivitamin preparations at concentrations ranging from 36 to 500 ppm (21 CFR 172). Together, these agents are approved for use in 35 different food products.

In addition to the direct additive uses of $CaNa_2EDTA$ and Na_2EDTA, EDTA and its calcium, ferric, potassium, and sodium salts are approved for indirect food additive uses as components of adhesives (21 CFR 175.105). In addition, Na_2EDTA is approved for use as a component of an aqueous sanitizing solution used on food-processing equipment and utensils (21 CFR 178.1010).

Use of EDTA compounds in the food supply considerably predates codification of the food additive regulations. EDTA salts were first developed in Germany in the 1930s as a substitute for citric acid. EDTA came into widespread use as a chelator in foods during the 1950s. By 1970, annual production of food-grade Na_2EDTA was 15,900 pounds and $CaNa_2EDTA$ was 75,000 pounds (NAS, 1989). By 1987, these figures had risen to 24,100 pounds and 162,000 pounds, respectively.

Nutritional efficacy of iron EDTA

Ferric (Fe^{3+}) food iron is precipitated from solution above pH 3.5. This insoluble precipitate is poorly absorbed in the upper small intestine by humans, where most non-heme iron is absorbed, unless suitable complexing agents are present (Conrad and Schade, 1968; MacPhail *et al.*, 1981).

The iron (primarily Fe^{3+}) in the meal remains complexed with EDTA under the acidic conditions prevailing in the stomach. The strength of the complex is progressively reduced as the pH rises in the upper small intestine, allowing partial exchange with other metals and the release of some of the iron for absorption. It has been demonstrated that iron chelated by EDTA (NaFeEDTA) is available for absorption via the physiologically regulated pathways for iron uptake, and that iron is dissociated from the EDTA complex before absorption (Candela *et al.*, 1984).

Human dietary fortification studies

Field trials in South and Central America, Thailand and South Africa have demonstrated that iron EDTA enhances iron absorption and reduces iron deficiency anaemia with no reports of adverse reactions.

Martinez-Torres *et al.* (1979) conducted a study using peasant volunteers from rural areas of Venezuela. The mean absorption of iron from iron EDTA ranged between 8 and 13%. The mean absorption from ferrous sulfate varied from 2 to 30% according to the food vehicle mixed with the salt. These results indicate that the iron absorption from the iron EDTA complex is only slightly affected by the presence of inhibiting substances, while iron absorption from ferrous sulfate is very sensitive to the inhibiting substances present in food vehicles.

Another study demonstrated increased absorption of both intrinsic and extrinsic food iron when iron EDTA complex was administered instead of other iron salts (Layrisse and Martinez-Torres, 1977).

Viteri *et al.* (1978) carried out iron EDTA absorption studies in Central America involving seven children and 98 adults to compare the proportion of iron absorbed when administered as ferric sulfate [$Fe_2(SO_4)_3$], iron EDTA and haemoglobin (Hb). These compounds were given with a milk–rice–sugar formula totalling 5 mg Fe to iron-deficient children. It was observed that iron from haemoglobin was absorbed best, followed by iron EDTA and by ferric sulfate (mean percent absorption $\pm$ SD = 34.5 ± 1.5, 8.6 ± 1.9 and 3.3 ± 1.5, respectively). Absorption of iron from iron EDTA was better than absorption from ferric sulfate in a standard meal providing a total of 5 mg Fe in normal and iron deficient adults. When iron EDTA and ferric sulfate are administered alone, the iron in iron EDTA is absorbed better than that in ferric sulfate and it improves the absorption of non-heme iron. Mean iron absorption from iron EDTA was 2.7 times higher than from ferric sulfate in 21 men studied in the Guatemalan lowlands. The 11 anaemic subjects did not absorb more than 10% and only three absorbed more than 4.5% of the iron from ferric sulfate. 10 subjects surpassed that level of absorption (10%) with iron EDTA, reaching values up to 19.9%. Another study by Viteri *et al.* (1995) examined population groups also in Guatemala, including one highland control community and three other communities (two in the lowlands and one in the highlands), in which iron EDTA fortified sugar was being administered. The impact of fortification on controlling iron deficiency was estimated at 8, 20 and 32 months of intervention. In the fortified communities, iron stores increased significantly in all populations except for women aged 18–44 years in one lowland community and age 45 years or over in the highland community. In the control community, the iron stores remained unchanged in children, females 18–44 years, and females 45 years or over, but increased significantly among adult males.

Iron EDTA was shown to be successful as an iron fortifying agent added to fish sauce in a pilot trial in Thailand (Garby and Areekul, 1974). Iron EDTA was added to fish sauce to a final concentration of 0.5 and 1.0 mg of elemental iron per ml of sauce. The addition of iron EDTA did not affect the visual appearance, pH or taste of the fish sauce. Packed red cell volume (PCV) and submaximal physical working capacity were measured before and 1 year after the treatment in both the test and control subjects. 1 year after the enrichment program, the mean change in the PCV in the test village ranged from +1.38 (for children) to +1.56 (for women); and the mean change ranged from −0.02 (for children) to +0.22 (for women) in the control village. An additional examination of the changes in submaximal working capacity and PCV values in 21 male subjects in the test village and four subjects in the control village was conducted at the beginning and at the end of the study. A significant increase in both mean PCV values and submaximal working capacity was observed in the test village but not in the control village. Over the course of the year study, 9% of the subjects in the test village became anaemic and 35% recovered from anaemia; whereas 16% of the subjects in the control village became anaemic and 20% recovered from anaemia.

A 2-year iron EDTA fortification trial was conducted in 264 single families from a South African population of Indian decent (Ballot *et al.*, 1989a,b). This was a controlled double-blind study where the subjects were randomly assigned to the control and test groups matched for iron status. Iron EDTA was added to the masala (curry powder) at a level of 25 μmol Fe/g masala; fortified or unfortified masala was distributed directly to each family each month free of charge. Iron status of the fortified group was shown to improve more than that of controls based on significant increases in haemoglobin levels, serum ferritin levels and body iron stores in fortified females and a significant increase in serum ferritin levels in fortified males. The prevalence of iron deficiency was significantly reduced in

fortified male and female groups, but the changes were more striking for women than men. Detected iron deficiency anaemia in women was reduced from 22.0% at the beginning of the study to 4.9% at the end. Fortified subjects did not accumulate excessive body iron and no alterations in serum zinc concentrations were found.

Human experimental studies

Candela *et al.* (1984) demonstrated that chelated iron from $Na^{59}Fe^{3+}$EDTA was available for absorption and that less than 1% of the dose administered was excreted as iron by the kidneys.

Studies with 127 Indian housewives given a rice diet fortified with either ^{59}Fe or ^{55}Fe-labelled $FeSO_4$ showed that addition of Na_2EDTA enhances iron absorption significantly in low bioavailability but not high bioavailability iron diets (MacPhail *et al.*, 1994). Both the intrinsic and added iron absorption were enhanced in the subjects by addition of EDTA. This enhancing effect on iron absorption was maximum when the EDTA:Fe molar ratio was 1 or less. Bioavailability of iron was particularly enhanced by Na_2EDTA in meals with low iron availability or with high levels of phytates and other substances that inhibit iron absorption.

In studies with 101 human females given $^{59}Fe^{3+}$EDTA added to corn porridge, iron absorption was increased twofold compared to porridge fortified with ferrous sulfate. Less than 1% of the radioactive iron from $^{59}Fe^{3+}$EDTA was excreted in urine (MacPhail *et al.*, 1981). Iron absorption was twice as high with the chelated iron in subjects who were fed ferrous sulfate one day and Fe^{3+}EDTA the next day. This finding suggests that the mechanism by which chelated iron forms a common pool with intrinsic iron differs from the mechanism of absorption for simple iron salts, resulting in greater overall iron bioavailability. Similar results were observed by Layrisse and Martinez-Torres (1977) in a study of 147 peasants from rural areas of Venezuela. The Fe^{3+}EDTA appeared to exchange completely with vegetable food iron in the gut. The absorption of both intrinsic and extrinsic iron was greater with Fe^{3+}EDTA than with other iron salts used for fortification.

Animal experimental studies

In swine, Candela *et al.* (1984) observed that the absorption of iron from $Na^{55}Fe[2\text{-}^{14}C]$EDTA was approximately 5% of the oral dose. The mucosal cells of the pylorus and the upper jejunum absorbed the ^{55}Fe, which was slowly transferred to plasma transferrin and incorporated into haemoglobin. A small proportion of the absorbed iron (less than 1% of the administered dose) was excreted by the kidneys, while the remaining 95% of the unabsorbed iron was excreted in the faeces. About 3% of the iron excreted in the faeces was in a soluble form, possibly bound to EDTA, and 92% was in an insoluble form. These results suggest that iron is dissociated from the EDTA moiety before its absorption and is absorbed via normal physiological pathways responsible for iron uptake.

Effect on other trace metals

Animal experimental studies

The dietary impact of food fortification with iron EDTA has been questioned on the basis of potential adverse effects on zinc and copper metabolism. The effect of EDTA on the absorption of other nutritionally important trace elements was studied in rats. The rats were fed either ferrous sulfate or iron EDTA with low zinc (6.1 mg/kg) soybean-based diets (Hurrell *et al.*, 1993). In rats fed a low zinc diet fortified with iron EDTA, zinc absorption was significantly increased and retention and elimination were enhanced in comparison with the same diet without iron EDTA. A significant increase in weight gain with increased zinc retention in animals receiving iron EDTA suggests that extra zinc was available for normal metabolic processes. Addition of either iron EDTA or Na_2EDTA to a zinc-sufficient diet resulted in increased absorption, urinary excretion and retention of zinc, but to a lesser extent than that seen in rats fed the zinc-deficient diet. Iron EDTA had very little effect on copper absorption and retention and in calcium elimination. The authors concluded that "using NaFeEDTA as a food fortificant would have no detrimental effect on the metabolism of zinc, copper and calcium and, in some circumstances, could improve zinc absorption and retention from low-bioavailability diets" (Hurrell *et al.*, 1993).

Similar increases in zinc bioavailability and improved animal growth were seen with turkey poults (Kratzer *et al.*, 1959), chicks (Scott and Ziegler, 1963) and rats (Forbes, 1961) when Na_2EDTA was used at 150–3000 mg/kg of feed to fortify a semi-purified soybean protein diet. The enhancing effect on zinc absorption is believed to be due to the formation of a soluble zinc chelate with EDTA that is potentially more bioavailable. EDTA prevents formation of zinc complexes with phytic acid that are not absorbed by humans.

Human experimental studies

Enhanced zinc absorption from low-bioavailability diets supplemented with iron EDTA as compared with $FeSO_4$ was found in a 6-day duration feeding study of 10 adult women (Davidsson *et al.*, 1994). This same study found no effect on calcium absorption and no effect on retention of zinc or calcium. In another study (Davidsson *et al.*, 1998), manganese absorption and urinary excretion were studied in adults after intake of a weaning cereal fortified with either iron EDTA or $FeSO_4$; no sig-

nificant differences were found in either absorption or excretion.

Toxicity review

Iron

Acute iron toxicity has typically resulted from accidental ingestion of medicinal iron or adult iron supplements by children. Severe toxicity is observed after the ingestion of approximately 2.5 g or more of ferrous sulfate (500 mg iron) (Klaassen, 1996). Signs of toxicity include lethargy, nausea and vomiting, abdominal pain, black stools, and signs of shock, as well as metabolic acidosis, liver damage, and coagulation defects that may occur several days after ingestion. Delayed effects include renal failure and hepatic cirrhosis.

Toxicity caused by long-term administration of iron and resultant iron overload (haemochromatosis) is more commonly seen in adults rather than children, but is generally limited to individuals with inherited metabolic disorders affecting maintenance of iron balance (e.g. human leucocyte antigen (HLA)-linked hereditary haemochromatosis) or excessive exposures (e.g. use of iron utensils in food and beverage preparation or repeated blood transfusions for the treatment of anaemia). The available evidence suggests that normal individuals are able to control the absorption of iron despite high intakes, and it is only individuals with underlying disorders that augment iron absorption who are at risk of developing haemochromatosis (Bothwell *et al.*, 1979; Hardman *et al.*, 1996; Klaassen, 1996).

EDTA and EDTA–cation complexes

As noted previously, metal ions on the EDTA–metal complex are freely exchanged once in the digestive tract. Accordingly, the various EDTA salt forms would be expected to have essentially similar toxicological potential and metabolic fate in the body, regardless of whether EDTA is consumed as the calcium, sodium or sodium–iron salt form (JNACG, 1993). Therefore, this review includes summaries of the toxicity of various EDTA–metal complexes as relevant to the assessment of the toxic potential of iron EDTA.

Because of the substantial differences in the availability of EDTA chelates administered by parenteral routes as compared with the oral route, this review focuses on studies performed by the oral route as most relevant to iron EDTA used as a food fortificant. Substantial information on the toxicological potential of various EDTA chelates administered by injection can be found in other reviews (JNACG, 1993; JECFA, 1993b).

Pharmacokinetics of orally-administered EDTA complexes. The EDTA moiety has been shown to be poorly absorbed following ingestion in humans. When ^{14}C-labelled CaEDTA was given orally to human subjects in a gelatin capsule, a maximum of 5% of the radioactivity associated with the EDTA was absorbed. Almost all of the administered dose was recovered unaltered in the faeces (91%) and urine (4%) within 3 days following administration (Foreman and Trujillo, 1954). Investigation of EDTA elimination following intravenous or intramuscular injection revealed that CaEDTA was excreted primarily in urine (> 98%) in the unaltered form, again indicating a lack of metabolism of this chelated product by humans (Foreman and Trujillo, 1954). ^{14}C-CaEDTA applied to human skin as a paste and occluded was minimally absorbed after 24 hr (maximum activity in urine was 0.001% of the applied dose) (Foreman and Trujillo, 1954).

The pharmacokinetics of CaEDTA in experimental animals is similar to that in humans. 88% of an orally administered dose of ^{14}C-labelled CaEDTA to rats appeared in the faeces and 10% in the urine after 24 hr. Less than 0.1% of the activity in the administered dose was found in respiratory CO_2, indicating that essentially none of the compounds were metabolized. Chromatographic analysis confirmed that the compound passed through the body essentially unchanged (Foreman *et al.*, 1953).

Human toxicity. The calcium disodium salt of EDTA ($CaNa_2EDTA$) is used clinically for the treatment of heavy metal poisoning (including lead and mercury poisoning) where the calcium is exchanged for the respective heavy metal, which is then mobilized and removed by the kidneys. EDTA has also been used for removing radioactive elements such as plutonium and strontium from humans exposed to radioactive materials and clinically for removal of copper in patients with Wilson's disease (Foreman *et al.*, 1953; Klaassen, 1996). Because most EDTA chelates are poorly absorbed from the gastrointestinal tract, chelation therapy is typically performed by parenteral administration (Klaassen, 1996). Kidney damage is a well characterized effect from overexposure to $CaNa_2EDTA$ or Na_2EDTA administered by parenteral injection for chelation therapy. Nephrotoxicity is similarly observed in animals given EDTA compounds by injection (Foreman *et al.*, 1956; Reuber, 1967; Reuber and Schmieler, 1962; Spencer, 1960). However, at lower exposure levels (e.g. those associated with its use as a food ingredient), nephrotoxicity has not been observed.

Field trials in South and Central America, Thailand and South Africa (Ballot *et al.*, 1989a,b; Garby and Areekul, 1974; Martinez-Torres *et al.*, 1979; Viteri *et al.*, 1978, 1995) involving dietary fortification with iron EDTA reported no adverse reactions to iron EDTA. (See section on nutritional efficacy of iron EDTA.)

Acute toxicity in animals. The oral LD_{50} of $CaNa_2EDTA$ in Wistar rats, rabbits, and dogs was 10,000, 7000 and 12,000 mg/kg bw, respectively (Oser *et al.*, 1963). The oral LD_{50} of Na_2EDTA in

Wistar rats was 2000–2200 mg/kg bw (Yang, 1952). Animals given higher doses of this salt of EDTA exhibited haemorrhages in the small intestine. Foreman *et al.* (1953) reported that single oral doses larger than 250 mg EDTA per animal caused diarrhoea in rats.

Subchronic toxicity in animals. Rats fed a diet containing 0, 0.5, 1.0 or 5.0% Na_2EDTA (equivalent to approximately 500, 1000, or 5000 mg/kg bw/day, respectively*) for 90 days exhibited no adverse effects except diarrhoea and reduced food consumption in the highest dose group only (Yang, 1952). Animals in the highest dose group and a comparable control group were litter mates from dams that had been fed a diet containing 0.5% EDTA for 8 months; animals in these groups were therefore exposed both pre- and postnatally.

Yang (1952) maintained groups of rats (three animals/sex/group) for 4 months on a low-mineral diet (one-half the normal salt concentration) containing 0 or 1.5% $CaNa_2EDTA$ (equivalent to approximately 1500 mg/kg bw/day*). The test group had a significantly reduced weight gain, but displayed no differences in their general condition.

Chan (1956) fed weanling albino rats a purified, low-mineral diet (0.54% Ca and 0.013% Fe) containing either 0.5 or 1.0% Na_2EDTA or $Na_2CaEDTA$ for 90 days (equivalent to approximately 500 or 1000 mg/kg bw/day, respectively*). Signs of diarrhoea and growth retardation were observed in the 1% Na_2EDTA dose group only. Extension of dosing for an additional 115 days (205 days total) resulted in growth retardation in the groups fed diets containing 1.0% of each EDTA chelate, and increased blood coagulation times, blood serum calcium levels, and an anaemic appearance in the group fed a 1.0% Na_2EDTA diet. Chan (1956) speculated that complexing of calcium in the blood may have interfered with the coagulation mechanism. Gross and histological examinations of the liver, kidney and spleen revealed no significant abnormalities. A substantial degree of dental erosion and lower ash content of bone was observed in animals receiving a low mineral diet with 1% Na_2EDTA, but not in rats fed a 1% Na_2EDTA diet with adequate mineral levels.

In a review of the studies by Yang (1952) and Chan (1956), BIBRA (1964) concluded that "the presence of minute quantities of Na_2EDTA (10–100 ppm) in foods would not be expected to have any significant effect on health", and that adult diets containing as much as 4000 ppm EDTA would still be far less than the lowest quantity fed to rats (i.e. 0.5% of the diet). They observed further that rats maintained on mineral-deficient diets with Na_2EDTA levels as high as 0.5% did not exhibit toxic effects.

Chronic toxicity in animals. Chronic toxicity studies in the rat, mouse and dog (NCI, 1977; Oser *et al.*, 1963; Yang, 1952) provide no evidence of adverse systemic effects associated with dietary administration of EDTA chelates.

In studies performed by the National Cancer Institute (NCI, 1977), male and female $B6C3F_1$ mice and F344 rats were fed diets containing 0, 3750 or 7500 ppm trisodium EDTA (Na_3EDTA) for 103 wk followed by a normal diet for 1 wk (equivalent to approximately 535 and 1070 mg/kg bw/day in mice, and to approximately 375 and 750 mg/kg bw/day in rats, respectively*). The Na_3EDTA diet was not associated with any adverse effects on clinical signs of toxicity, survival or incidence of neoplastic or non-neoplastic lesions. There was a significant dose-related increase in survival of female rats, and a slight dose-related depression in bw gain in mice only.

Yang (1952) extended the 90-day subchronic rat feeding study with 0, 0.5 or 5% Na_2EDTA in the diet for a total period of 2 years. No effects were noted in growth, survival, food consumption, blood coagulation times, red cell counts, or in ash content of bones in comparison to control groups. Histopathological examination revealed no treatment-related effects. The diarrhoea and reduced food consumption reported after 90 days in the 5% dose group were apparently no longer observed after 2 years on the diet.

Four groups of mongrel dogs (four animals/group) were fed diets designed to provide 0, 50, 100 or 200 mg $CaNa_2EDTA$/kg bw/day for 12 months (Oser *et al.*, 1963). No adverse effects were noted in appearance, health status, haematology, urinalysis or blood chemistry parameters, bone or dental calcification, or gross and histopathologic findings.

Reproductive and developmental toxicity in animals. Oser *et al.* (1963) maintained Wistar rats (25 animals/sex/group) on nutritionally adequate diets (i.e. standard laboratory feed supplemented with inorganic salts, vitamins and cottonseed oil) and adjusted to provide 0, 50, 125 and 250 mg $CaNa_2EDTA$/kg bw/ day for up to 2 years. Feeding was carried out through four successive generations (F_0, F_1, F_2, F_3) to examine potential adverse effects on reproduction and lactation. 10 rats from each treatment group representing the F_1 generation were raised to maturity and allowed to produce two litters; a similar regimen was followed for the F_2 and F_3 litters. The second litters from each gener-

*Where dosage conversions from ppm diet to mg/kg bw/day were not provided by the study investigators, approximate dosage conversions were performed using the following factors from Derelanko and Hollinger (1995):

Species (and age)	Conversion factor (divide ppm by:)
Mouse (older)	7
Rat (young)	10

ation (control and 250 mg/kg bw/day groups only) were maintained without change in dietary treatment until the end of the 2-year period for the F_0 generation. This protocol permitted observations to be made on rats receiving test diets for 0.5, 1, 1.5 or 2 yr in the F_3, F_2, F_1 and F_0 generations, respectively. Investigators reported no treatment-related differences in growth, survival, gross or histopathology, reproductive performance, or haematology, urinalysis or blood chemistry parameters when animals were fed up to 250 mg/kg bw/day $CaNa_2EDTA$ in the diet (reported by the authors to be equivalent to a dietary concentration of 5000 ppm) for four generations.

Swenerton and Hurley (1971) fed pregnant female Sprague–Dawley rats a purified diet containing 2 or 3% Na_2EDTA (equivalent to approximately 2000 or 3000 mg/kg bw/day, respectively*) and 100 ppm zinc carbonate during gestation. All females fed Na_2EDTA had moderate to severe diarrhoea. The 2% Na_2EDTA diet caused a slight impairment of reproduction and an increased incidence of malformations (including brain, cleft palate, malformed digits, clubbed legs and malformed tails). The 3% Na_2EDTA diet severely affected reproduction. In a second study, pregnant females were fed either the control diet with 100 ppm zinc, 3% Na_2EDTA with 100 ppm zinc or 3% Na_2ETDA supplemented with 1000 ppm zinc on gestation days 6–21. Significant increases in malformations were seen in animals given 3% Na_2EDTA at the low zinc level of 100 ppm but not with 1000 ppm zinc. The investigators suggested that the observed teratogenic effects were not attributable to EDTA itself but rather to an induced deficiency of zinc that was insufficient to support normal foetal development.

Kimmel (1977) demonstrated that the route of administration of Na_2EDTA had a marked effect on reproductive and developmental toxicity. Groups of pregnant rats were given Na_2EDTA in a semipurified diet at a dose of 954 mg/kg bw/day (3% in diet); by gavage at a dose of 1250 mg/kg bw/day (given as a split dose of 625 mg/kg bw twice/day) or 1500 mg/kg bw/day (given as a split dose of 750 mg/kg bw twice/day); or by subcutaneous injection at a dose of 375 mg/kg bw/day. Animals in all groups were dosed on gestation days 7 through 14 and foetuses were examined on gestation day 21 for skeletal and visceral malformations. Maternal toxicity was evident by decreased food consumption, diarrhoea, and reduced weight gain in groups treated by all three dose routes. Dietary administration of EDTA resulted in no maternal deaths, but malformations were seen in 71% of the offspring. By gavage, EDTA was more toxic to the dams, but resulted in fewer malformed offspring (20.5% at 625 mg/kg bw/day) than did dietary administration. By subcutaneous injection (at a dose about one-third to one-half that of the other routes), EDTA was lethal to 24% of the dams, but produced a low rate (4.3%) of malformed offspring. Kimmel (1975, 1977) and Kimmel and Sloan (1975) speculated that the mechanism by which EDTA induced developmental toxicity was the binding of zinc by EDTA resulting in zinc deficiency during embryonic development. In support of this proposed mechanism, Kimmel noted that the observed malformations were similar to those seen in zinc-deficient rats, and that the findings of their laboratory were consistent with Swenerton and Hurley (1971) when a low-zinc diet was used.

Shardein *et al.* (1981) investigated the developmental toxicity of EDTA (acid form), Na_2EDTA, Na_3EDTA, Na_4EDTA or $CaNa_2EDTA$. Equimolar doses of 1000 mg/kg bw/day were given in two split doses by gavage on days 7–14 of gestation to groups of 20 pregnant rats that were maintained on a nutritionally complete, standard lab chow diet. Dams were sacrificed on day 21 of gestation and foetuses were examined for abnormalities. Diarrhoea and decreased food intake and weight gain were observed in all treated groups. EDTA, Na_2EDTA, and Na_4EDTA had the greatest effects on weight gain. None of the test compounds significantly affected litter size, foetal mortality, or rate of malformations. The absence of positive findings in this gavage study contrasts with the high incidence of malformations noted by Kimmel (1977) in gavage studies performed with animals receiving a semipurified diet with limited levels of zinc and other micronutrients.

The studies summarized above reveal a lack of reproductive and developmental toxicity associated with EDTA compounds when administered orally along with nutritionally adequate diets (Oser *et al.*, 1963; Shardein *et al.*, 1981), or when purified diets were fortified with zinc (Swenerton and Hurley, 1971).

Genotoxicity. A review of the literature by Heindorff *et al.* (1983) indicated that EDTA inhibited DNA synthesis and repair and produced a low degree of chromosomal damage and gene mutations *in vitro*. FDA scientists (Lerner *et al.*, 1986) concluded that the observed events were probably spurious indications of genotoxic potential caused by the chelation of cations that are important as enzymatic cofactors involved in DNA synthesis in the cell. Taylor and Jones (1972) reported that transient inhibition of DNA synthesis was observed in rat

*Where dosage conversions from ppm diet to mg/kg bw/day were not provided by the study investigators, approximate dosage conversions were performed using the following factors from Derelanko and Hollinger (1995):

Species (and age)	Conversion factor (divide ppm by:)
Mouse (older)	7
Rat (young)	10

kidneys following intraperitoneal injection of various calcium, sodium or manganese chelated forms of EDTA, but not by zinc salts of EDTA. These authors speculated that the lack of effects by the zinc EDTA salt provided evidence that zinc is required for the initiation or continuation of DNA synthesis and that the other EDTA salts probably caused a depletion of the required zinc ions in the kidney tissues. The importance of zinc in tissues undergoing rapid growth and cell division was also noted in the developmental studies of Kimmel (1975) and Kimmel and Sloan (1975). Na_3EDTA was not mutagenic in *in vitro* assays with *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537, TA1538) or with *Escherichia coli* WP uvrA (Dunkel *et al.*, 1985). The L5178Y mouse lymphoma cell forward mutation assay (McGregor *et al.*, 1988), either with or without a rat liver S9 metabolic activation system, showed no mutagenic activity.

A recent publication by Dunkel *et al.* (1999) reported that of 11 forms of iron salts tested in the Ames *Salmonella* assay with or without S9 metabolic activation, only ferrous fumarate produced slight increases above control levels and iron EDTA (NaFeEDTA) and other salts were not mutagenic. In the L5178Y mouse lymphoma assay, in contrast to negative findings by McGregor *et al.* (1988), positive increases in mutants were obtained with most of the iron compounds. Iron EDTA produced a concentration-related, but only a marginally positive, increase of 2.5–2.7-fold over control values at the highest dose tested in the presence and absence of a rat liver S9 metabolic activation system, respectively. At the highest concentrations that were considered mutagenic, increases in mutant incidence above control values for common iron compounds were approximately 3.2-fold for ferrous sulfate either with (+) or without (–) S9; 2.8 (+) and 2.0 (–) for ferric chloride; and 2.5 (+) and 1.3 (–) for ferric phosphate. Ferrocene complex was not mutagenic with S9 but produced 7.2-fold increases above control values without S9. The absence of mutagenicity in the Ames test for the same compounds suggests that mouse lymphoma cells may be particularly sensitive to incorporation of excessive quantities of iron salts in the tissue culture growth medium. The significance of mutations produced by iron compounds added at non-physiological concentrations in an *in vitro* screening system is difficult to extrapolate for relevance to intact organisms. As the finding with iron EDTA is typical of the other irons salts tested, and was not the highest response observed, it most likely reflects the sensitivity of the L5187Y cells to abnormal iron concentrations. Thus, the finding with iron EDTA is not unique and it is of minor importance in the overall assessment of its safety.

The overall findings indicate that EDTA–metal complexes lack significant genotoxic potential under conditions that do not deplete essential trace elements required for normal cell function.

Allergenicity. Na_2EDTA was evaluated for potential to produce an allergic response in male guinea pigs administered on alternate days 10 injections of 0.1 ml of a 0.1% Na_2EDTA solution (Yang, 1952). No sign of allergenicity was noted after a challenge injection of a 0.1% solution given 2 weeks after the last injection. In a human skin-sensitization study with $CaNa_2EDTA$, Raymond and Gross (1969) reported a positive response in 3/50 individuals that cross-reacted with ethylenediamine. They concluded that given the widespread experience with $CaNa_2EDTA$ and the absence of reports of adverse reactions, it was at most a weak sensitizing agent.

Evaluations by expert bodies

In 1974, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974) recommended that $CaNa_2EDTA$ or Na_2EDTA be permitted as food additives at doses up to an ADI of 2.5 mg EDTA/kg bw/day, based on the facts that these additives are poorly absorbed from the gut, appear to be metabolically inert, and have a history of use in treating metal poisoning in humans.

Subsequently, the International Nutritional Anemia Consultative Group (INACG, 1993) and JECFA (1993b,c) evaluated the benefits and potential health concerns associated with the use of iron EDTA as an iron fortificant in food. Among their conclusions were the following:

- Iron EDTA in the concentration range intended for food fortification would not have toxic side-effects based on studies in animals and humans. The EDTA moiety is minimally absorbed (5% or less) and the fraction absorbed is excreted by the kidneys unchanged after 24 to 48 hr. The widespread use of Na_2EDTA and $CaNa_2EDTA$ approved by the FDA for use by the food industry has not been associated with any adverse effects.
- Iron and EDTA disassociate in the gastrointestinal tract prior to absorption by the mucosal cells and they display independent absorption processes. Sufficient information on the safety of these compounds is available to demonstrate their safety at proposed fortificant concentrations and further toxicological research is not required.
- Concern over the use of iron EDTA for prolonged periods of time in the diet because of trace metal depletion appears to be unfounded. Exchange of iron for zinc is the predominant reaction of concern because of its potential effect on zinc balance. Effects on zinc balance depend on the EDTA:zinc ratio, and the proposed iron EDTA concentration range for fortification would not be expected to have detrimental effects on zinc balance.

- Absorption studies in humans show that iron in iron EDTA is absorbed two to three times higher than ferrous sulfate and that iron EDTA enhances the absorption of intrinsic iron from low-bioavailability meals.
- Three food fortification trials with human volunteers have demonstrated that iron EDTA has a significant beneficial effect on iron status.

JECFA "provisionally concluded that use of sodium iron EDTA... would not present a safety problem in supervised food fortification programmes in iron-deficient populations", and referenced in their safety evaluation the ADI of 2.5 mg EDTA/kg bw/day for disodium and calcium disodium salts of EDTA published by JECFA in 1974 (JECFA, 1993c).

In 1999, JECFA reviewed additional data on iron EDTA and removed the "provisional" qualification from the 1993 review. The Committee "concluded that sodium iron EDTA could be considered safe when used in supervised food fortification programmes..." (JECFA, 1999).

In the US, the GRAS process ensures that proposed uses of iron EDTA are reviewed for safety before they are implemented. Further, the existence of a sophisticated National Nutrition Monitoring System that includes large-scale surveys of the food consumption behaviour of the US population ensures that the potential exposure of the population and population subgroups to iron EDTA from actual or proposed uses can be accurately assessed. Finally, the intended uses of iron EDTA do not call for any increase in the level of iron fortification from that already existing and are in full compliance with FDA's Fortification Policy (21 CFR 104.20). For these reasons the use of iron EDTA in the US may be regarded as occurring within a "supervised food fortification programme."

Specifications for food grade material

The Kellogg Company analysed six batches of food-grade iron EDTA produced by Lohmann GmbH. A summary of these analytical results and food grade specifications is presented in Table 1.

Exposure assessment

Concentration of iron EDTA

Iron EDTA is intended for use as a fortificant in four grain-based products: (1) ready-to-eat (RTE) breakfast cereals, (2) toaster pastries, (3) cereal-based breakfast bars and (4) granola bars. These products are currently fortified with iron in compliance with the Food and Drug Administration's Fortification Policy Guidelines (21 CFR 104.20). Iron EDTA is intended to provide an alternative compound to those currently available. Iron EDTA is intended to provide a maximum of 20% of the daily value (DV) for iron, or 3.6 mg iron/serving in RTE cereals, toaster pastries and breakfast bars, and 15% of the DV, or 2.7 mg iron/serving, in granola bars because of their smaller serving size. [The DV for iron established by NRC (1989) is 18 mg iron/person/day.] Any additional iron fortification above 20% DV would be provided by alternative iron compounds.

The amount of iron EDTA to be added to foods is based on the target level for iron. The corresponding amounts of EDTA and complete iron EDTA complex to be added to the food products

Table 1. NaFeEDTA. Six-batch analyses summary

Product property	Product specifications	Average of batches
Assay tests		
Fe (%)	NLT 12.5%, NMT 13.5%	13.6
EDTA (%)	NLT 65.5%, NMT 70.5%	69.0
Water (%)	N/A	13.0
Na (%)	N/A	5.62
Identity tests		
Fe	+ test	+
Water solubility (%)	Soluble	10% @ 25°C
Purity tests		
pH (1% soln)	3.5–5.5	4.8
Heavy metals		
As (ppm)	NMT 3 ppm	0.4
Pb (ppm)	NMT 1 ppm	< 0.5
Hg (ppm)	N/A	0.03
Zn (ppm)	NMT 25 ppm	3.0
Cu (ppm)	NMT 10 ppm	< 9.0
Cd (ppm)	N/A	< 0.05
Water insoluble matter (%)	NMT 0.1%	≤0.1
Nitrilotriacetic acid (NTA) (%)	NMT 0.1%	< 0.1
Manufacturing/acceptance tests		
Chloride (Cl^-) (%)	NMT 0.1%	≤0.05
Sulfate (SO_4^{2-}) (%)	NMT 0.2%	≤0.2
Mg (ppm)	NMT 400 ppm	< 140
P (ppm)	NMT 400 ppm	< 140
K (ppm)	NMT 2000 ppm	< 700

NLT = not less than; NMT = not more than.

can be calculated as follows. The specifications for the trihydrate form of iron EDTA require that iron comprises 12.5 to 13.5% of the fortificant by weight and 65.5 to 70.5% EDTA. Thus, the maximum EDTA content (70.5%) will occur in lots with the minimum iron content (12.5%). At most, then, there will be 70.5/12.5 or 5.64 times as much EDTA as iron in a food product. Similarly, because iron constitutes at least 12.5% of the material, the maximum complete trihydrate iron EDTA content will be 100/12.5 or 8.0 times the level of iron.

Serving sizes for foods to which iron EDTA would be added range from 21 g for granola bars to 50 g for toaster pastries. Based on serving size and target iron levels per serving, the concentration of the fortificant in a given product can be calculated (Table 2).

Estimated daily intakes from intended food uses

EDIs for the intended uses of iron EDTA were estimated for the total US population and for two subpopulations with relatively high intakes of the food products to which iron EDTA will be added (i.e. children age 1–6 years, with high daily intakes of food per kg bw, and males age 13–19 years, with high daily intakes of food per person). These intakes were estimated using the TAS International Diet Research System (TAS-DIET™) (TAS, 1995) and food consumption data from the 1989–91 Continuing Survey of Food Intakes by Individuals (89–91 CSFII) conducted by the Agricultural Research Service of the US Department of Agriculture (ARS, 1994).

As suggested by the Food and Drug Administration, which defines "heavy" consumers as those individuals who consume the food at or above the 90th percentile of the food intake distribution (DiNovi and Kuznesof, 1995), EDIs for each food product and for all four food products combined were based on the 90th percentile of intake per user. Further, the "worst case" assumption was made that the four fortified products captured 100% of the market such that all brands of RTE breakfast cereals, toaster pastries, breakfast bars and granola bars consumed by all individuals contain iron EDTA at the maximum concentration level.

EDIs for iron from intended and current uses combined. The EDI for iron in the total population, assuming use of all four food products combined, is 3.93 mg iron/person/day. This compares with the current intake value of 5.76 mg iron/person/day.

EDIs for EDTA from intended uses. Projected 90th percentile daily intakes of EDTA for the US population as a whole and for the two subpopulations are summarized in Table 3.

EDIs of EDTA from current uses. As previously noted, Na_2EDTA and $CaNa_2EDTA$ are approved for direct food-additive use in 35 different food products (21 CFR 170) at concentrations ranging from 25 to 800 ppm (21 CFR 170; Whittaker *et al.*, 1993).

Whittaker *et al.* (1993) estimated that mean EDTA exposure from consumption of regulated foods did not exceed 15.12 mg EDTA/person/day or 0.25 mg EDTA/kg bw/day for a 60-kg person. Whittaker *et al.* (1993) regarded this estimate as an upper bound, overstating both food intake and exposure to EDTA. Comparison of Whittaker's EDTA exposure estimate to a *per capita* estimate based on the amount of EDTA available for use in the US food supply suggests that this estimate is indeed high. Based on National Academy of Sciences figures for the total poundage of food-grade EDTA salt for 1987 of 186,000 pounds (NAS, 1989), *per capita* intake is estimated to be 1.6 mg/person/day, or one-tenth Whittaker's estimate.

The Whittaker analysis was refined with respect to intake estimates for dry pinto beans and black-eyed peas, for additional uses of $CaNa_2EDTA$ approved since the Whittaker analysis was conducted, and for elimination of exposure from cola beverages that no longer use $CaNa_2EDTA$ in coated cans in the United States. This refinement resulted in an estimated intake of EDTA from all regulated food sources of 7.75 mg EDTA/person/day, or 0.13 mg EDTA/kg bw/day (for a 60-kg person).

EDIs of EDTA from current uses and intended uses combined. For the total population, the 90th percentile intake of EDTA from both current and intended uses is estimated to be 1.15 mg EDTA/kg bw/day (i.e. 1.02 mg/kg bw/day +0.13 mg/kg bw/

Table 2. Proposed concentration levels of iron EDTA

Food group	Serving size (g)	Iron/serving (% DV)	Iron/serving (mg)	Maximum fortificant concentration (ppm)		
				Iron[a]	EDTA[b]	Iron EDTA[c]
RTE breakfast cereals	28	20	3.6	127	716	1016
Toaster pastries	50	20	3.6	72	406	576
Cereal-based breakfast bars	37	20	3.6	97	547	776
Granola bars	21	15	2.7	128	723	1026

[a]Iron per serving ÷ serving size.
[b]Iron concentration × 5.64.
[c]Iron concentration × 8.0.

Table 3. 90th Percentile intake estimates

	US Population		13–19-year old males	1–6-year-old children
	mg/person/day	mg/kg bw/day	(mg/person/day)	(mg/kg bw/day)
EDTA	44.33	1.02	55.23	1.93
Iron EDTA	62.88	1.47	74.08	2.74

Table 4. Estimated intakes of iron and EDTA in field trials of iron EDTA

Study	Iron intake (mg Fe/person/day)	EDTA intake[1] (mg EDTA/person/day)
Thailand—fish sauce (Garby and Areekul, 1974)	10–15	56.4–84.6
Central America—sugar (Viteri *et al.*, 1983)	4.3	24.3
South Africa—masala (Ballot *et al.*, 1989a,b)	7.7	43.4

[1]Calculated as 5.64 × Fe.

day). The highest potential intake of EDTA is among young children (age 1–6 years). For this group, the mean intake of EDTA from current plus intended uses is estimated to be 1.08 mg EDTA/kg bw/day, and the intake at the 90th percentile is estimated to be 2.06 mg EDTA/kg bw/day (i.e. 1.93 mg/kg bw/day + 0.13 mg/kg bw/day).

Safety evaluation

To evaluate the safety of iron EDTA, EDIs for EDTA were compared to the ADI of 2.5 mg EDTA/kg bw/day established by FAO/WHO (JECFA, 1974). Comparison of 90th percentile intakes from the intended food uses of iron EDTA for the US population (1.02 mg EDTA/kg bw/day) and for children aged 1–6 years (1.93 mg EDTA/kg bw/day) reveals that the EDIs are 41% and 77% of the ADI, respectively. A similar comparison based on both current and intended uses combined reveals that the EDIs are 46% and 82% of the ADI, respectively. Thus, even at the 90th percentile of intake, in no case does the intended use of iron EDTA result in an EDI that exceeds the ADI. This is true despite the fact that the analyses are based on the assumption that iron EDTA will be used at maximum intended concentrations in all brands and all product lines of RTE cereals, toaster pastries, breakfast bars and granola bars, as well as in all currently approved applications. Therefore, these analyses represent substantial overestimates of likely actual exposure to iron EDTA.

As previously noted, toxicological studies of iron EDTA itself have not been conducted; rather, the ADI for EDTA was determined based on safety studies and the history of use of other EDTA compounds. Thus, it is particularly important to note that the estimated mean intakes of iron and EDTA from the intended uses are comparable to or lower than those used in the three large-scale field trials of iron EDTA as a fortificant (Table 4).

The intended applications of iron EDTA as a fortificant result in estimated mean intakes of iron of 3.93 mg Fe/person/day for the total population, 2.90 mg Fe/person/day for children age 1–6 years, and 5.14 mg Fe/person/day for teenage males. The estimated intake of EDTA that will result from the intended uses of iron EDTA is 22.17 mg EDTA/person/day for the US population, 16.36 mg EDTA/person/day for children age 1–6 years, and 28.99 mg EDTA/person/day for teenage males.

In summary, all exposure estimates are well below the JECFA ADI, and independent examination of the currently available toxicology literature provides no evidence of potential hazard under intended use conditions. The data collected and published over the past 20 to 30 years demonstrate that iron EDTA is safe and effective for iron fortification of food products and meets the standard of "reasonable certainty of no harm." Further, the published record establishes that the safety of iron EDTA for its intended use may be regarded as a matter of general recognition.

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Preventing Iron Deficiency Through Food Fortification

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Richard F. Hurrell, Ph.D.

One way to prevent iron deficiency anemia in developing countries is through the fortification of food products with iron. In addition to avoiding undesirable color and flavor changes, the main challenge is to protect the fortification iron from potential inhibitors of iron absorption present in commonly fortified foods.

Introduction

There is clear evidence of a high prevalence of iron deficiency anemia in developing countries and, to a lesser extent, in the more industrialized countries of the world. Most critically affected are infants, school-age children, and women of reproductive age. Approximately 50% of these populations suffer from anemia in the less-developed countries of South Asia and Africa, compared with about 25% in Latin America and approximately 10% in the industrialized countries of Europe.[1] In addition to the deleterious physiologic consequences of iron deficiency in individuals, the resulting public health consequences in developing countries can significantly impact economies in the form of health costs, wasted educational resources, and lost productivity.

Before considering an intervention strategy to prevent iron deficiency, its etiology must be understood. This is more complex in developing countries than in industrialized countries where the consumption of insufficient absorbable iron is usually the only cause or may be the major factor causing iron deficiency.[2] In developing countries, other possible causes are intestinal worm infections, malaria, and vitamin A deficiency.[3,4] The major causative factor in developing countries is not low iron intake, but, rather, low iron absorption. Iron intake is often relatively high, almost 20 mg/day,[3] and would easily meet the recommended dietary allowances for the United States (10–15 mg/day).[5] Unfortunately, much of the ingested iron is poorly bioavailable iron from plant sources or is contamination iron from soil and includes little bioavailable iron from animal tissues. Major cereals, legumes, and staple foods contain high levels of phytic acid, which is a potent inhibitor of iron absorption,[6,7] and some, such as sorghum, also contain phenolic compounds, which greatly impede iron absorption[8] by binding iron in the gut in unabsorbable complexes. The intake of foods that enhance iron absorption such as fruits and vegetables containing vitamin C[9] or muscle tissue[10] is often limited.

The fortification of foods is often regarded as the most cost-effective long-term approach to reducing the prevalence of iron deficiency.[11,12] This can be in the form of "mass medication" by fortifying foods such as cereals, milk, salt, and condiments that are widely consumed by both at-risk populations and others who have little or no need for extra iron. Alternatively, a targeted fortification program in which a food product preferentially consumed by one of the at-risk groups is fortified can be considered.

Although targeted fortification is relatively easy to design for infant foods such as formulas and commercial infant cereals, or for schoolchildren through school feeding programs including such foods as fortified drinks or cookies, it is more difficult to target a fortified food specifically for adult fertile women. For this group, the fortification of a widely consumed product would seem the best way to provide extra food iron, but other groups such as adult men and postmenopausal women, who do not require extra iron, will also consume the fortified food. In industrialized countries, there is concern that this excess iron may be detrimental and lead to increased incidence of atherosclerosis[13] and cancer[14] owing to increased oxidative stress.

In developing countries, however, where a lower intake of bioavailable iron occurs, these considerations might not apply. The prevalence of anemia in adult men has been reported to range from 2% in Europe and 4% in North America, to 13% in Latin America, 20% in Africa, and 32% in South Asia.[1]

Although widespread iron deficiency has been recognized for more than 50 years, intervention strategies including food fortification have been met with limited success. The only clear success story has been in industrialized countries, such as the United States and Sweden, where the steady drop in the prevalence of iron deficiency in infants and preschool children over the last 30 years[15] is

Dr. Hurrell is Professor of Human Nutrition at the Institute of Food Science, Laboratory for Human Nutrition, Swiss Federal Institute of Technology, Zürich, CH-8803 Rüschlikon, Switzerland.

considered to be the result of the consumption of iron-fortified infant formulas.[16] The current low incidence of iron deficiency anemia in fertile U.S. women, reported as 2.9% in the NHANES II survey,[17] could also be due in part to the high consumption of iron-fortified foods in the United States.[12] Fortification of products such as white bread, rolls, crackers, corn flour, corn grits, pasta, and breakfast cereals is widespread, and fortified iron from these products represented about 20% of the total iron intake as determined in NHANES II.[18,19]

Although pilot fortification trials in developing countries have given promising results, there are as yet no major success stories except perhaps for Chile.[20,21] This could be due to lack of political commitment, insufficient funding, too little technical support from local or multinational industries, poor distribution networks, or lack of nutrition education programs for the consumer, all of which are considered necessary for a successful fortification program.[22] Another reason could be that iron deficiency is due to factors other than insufficient absorbable iron. Hookworm infections, malaria, and vitamin A deficiency should be addressed simultaneously in any food fortification strategy. However, if low bioavailability of food iron is the major determinant of iron deficiency anemia in developing countries,[23] increasing the supply of absorbable food iron should decrease the prevalence of iron deficiency anemia.

This necessitates the careful selection of both the food product to be fortified and the iron fortification compound to be added. Clearly, the iron compound must be first optimized with respect to relative bioavailability.[24] However, if the food vehicle contains potent inhibitors of absorption, the added iron, like the native iron, will be poorly absorbed and will have little or no impact on the iron status of the consumer. The success of a food fortification program thus depends heavily on the absorbability of the added iron and its protection from major dietary absorption inhibitors.

This review focuses on the technical aspects governing the choice of food vehicle and iron compound with the aim of ensuring an adequate absorption of fortification iron. The optimization of the iron compound in relation to bioavailability and organoleptic problems is discussed first, followed by a description of methods that can be used to protect fortification iron from absorption inhibitors. These include the addition of ascorbic acid, the use of hemoglobin or dried blood, and the use of NaFeEDTA. Finally, the major foodstuffs that are used as iron fortification vehicles are discussed in relation to potential organoleptic problems, the presence of absorption inhibitors, and possible fortification compounds.

Optimization of the Iron Compound

Some characteristics of commonly used iron compounds are shown in Table 1.[12,24,25] They can be conveniently divided into four groups: (1) those that are freely water-soluble; (2) those that are poorly water soluble but soluble in dilute acids such as gastric juice; (3) those that are water

Table 1. Characteristics of Iron Sources Commonly Used to Fortify Food (adapted from Hurrell 1985, 1992; Bothwell & McPhail 1992)

	Approximate Fe content (%)	Average relative bioavailability		Approximate relative cost[a]
		Rat	Man	
Freely water soluble				
Ferrous sulfate $7H_2O$	20	100	100	1.0
Dried ferrous sulfate	33	100	100	0.7
Ferrous gluconate	12	97	89	5.1
Ferrous lactate	19	—	106	4.1
Ferric ammonium citrate	18	107	—	2.1
Poorly water soluble/soluble in dilute acid				
Ferrous fumarate	33	95	100	1.3
Ferrous succinate	35	119	92	4.1
Ferric saccharate	10	92	74	5.2
Water-insoluble/poorly soluble in dilute acid				
Ferric orthophosphate	28	6–46	25–32	4.1
Ferric ammonium orthophosphate (EKA Nobel, Sweden)	19	—	30–60	—
Ferric pyrophosphate	25	45–58	21–74	2.3
Elemental Fe powders: electrolytic	98	44–48	5–100	0.5
carbonyl	98	39–66	5–20	1.0
reduced	97	24–54	13–148	0.2
Protected compounds				
NaFe EDTA	14	—	28–416	6.0
Hemoglobin	0.34	—	100–700	—

Adapted from references 10, 12, and 25.
[a]Relative to ferrous sulfate $7H_2O$ = 1.0, for the same level of total iron.

insoluble but poorly soluble in dilute acid; and (4) protected iron compounds. The table gives guideline values for relative bioavailability in rat and man and a relative cost factor. A more detailed description of these and other compounds can be found in reference 25.

The cost of the more recent or experimental compounds such as NaFeEDTA, ferric ammonium orthophosphate,[26] and hemoglobin depends to some extent on the amounts ordered. In general, the freely water-soluble compounds are highly bioavailable in rodents and humans, as are compounds that are water insoluble but soluble in dilute acids. Compounds that are poorly soluble in dilute acid, however, have only a low to moderate bioavailability. This is because of variable dissolution in gastric juice owing to both the characteristics of the compound itself[27] and the meal composition.[28] Although it would be logical to always use iron compounds of highest bioavailability, they unfortunately often cause unacceptable color and flavor changes in many foods. Optimization, therefore, means selecting the iron compound with the highest potential absorption without causing subsequent organoleptic problems in the food vehicle.

Bioavailability

The absorption of fortification iron depends primarily on its solubility in gastric juice. Water-soluble compounds such as ferrous sulfate dissolve instantaneously in gastric juice, whereas more insoluble compounds, such as elemental iron, rarely dissolve completely. Once dissolved, fortification iron enters the common pool, where its absorption (like that of all pool iron) depends on the content of enhancing or inhibitory ligands in the meal and on the iron status of the subject. For example, phytate and polyphenols or a satisfactory iron status in an individual will diminish absorption, whereas vitamin C or low iron status will enhance absorption.

Because iron status and various food components may markedly affect iron absorption, the absorption of a single iron compound can vary from less than 1% to almost 100%. Therefore, when comparing different iron compounds, one must measure the bioavailability relative to a standard compound. The standard is usually ferrous sulfate, which has been designated as having a relative bioavailability (RBV) of 100. It has recently been demonstrated that the hemoglobin repletion test in rodents and the measurement of dialyzable iron in vitro are good predictors of iron bioavailability in humans.[29] The RBV of many commercial iron compounds is well known (Table 1[12,24,25]). New compounds can be screened by animal or in vitro assays, although human studies are ultimately necessary.

Compounds labeled with radioactive or stable isotopes can be prepared and used as confirmation for the more soluble compounds. For those compounds that are poorly soluble in dilute acids, however, such as phosphate and elemental iron powders, one is never absolutely sure that the labeled experimental compound made on a small scale has exactly the same physiochemical characteristics as the commercial compound.[29] The best confirmation of the utility of these compounds is intervention studies monitoring iron status.[30]

Organoleptic Problems

In addition to causing unacceptable changes in color and flavor when added to foods, iron compounds may also provoke precipitation, such as when added to fish sauce[31] or when iron-fortified sugar is added to tea.[32] Many iron compounds are colored and cannot be used to fortify light-colored foods. In addition, the more soluble iron compounds often react with substances in foods, causing discoloration. Infant cereals have been found to turn gray or green on addition of ferrous sulfate and dark blue if bananas are present.[27] Phenolic compounds have often been implicated, and Douglas et al.[33] reported that ferrous sulfate, ferrous lactate, ferrous gluconate, and ferric ammonium citrate, as well as the less soluble ferrous fumarate and ferric citrate, produce off-colors when added to a chocolate milk drink. Similarly, salt fortified with ferrous sulfate or other soluble iron compounds becomes yellow or brown.[34]

Off-flavor can also result from the metallic taste of the soluble iron itself, particularly in beverages. However, the catalytic effect of iron on fat oxidation in cereals during storage is the major problem. As in the case of product discoloration, the water-soluble compounds, such as ferrous sulfate, promote fat oxidation and reduce product shelf life. A convenient method to measure the potential of iron fortification compounds to promote fat oxidation in cereals is to measure pentane formation in the headspace of sealed cans containing the iron-fortified product.[35]

Pentane is the major hydrocarbon formed by the oxidative degradation of linoleic acid, and its formation correlates with the production of off-flavors. Figure 1 shows the rate of pentane formation during storage at 37 °C of a precooked whole wheat flour containing various iron salts (at a concentration of 15 mg iron per 100 g flour).[27] Ferrous sulfate and ferrous gluconate rapidly generated pentane and were judged unacceptable by a taste panel after 4 to 6 weeks of storage. Ferric pyrophosphate and reduced elemental iron generated far less pentane and were still organoleptically acceptable after 7 weeks of storage. A similar oxidative rancidity can occur in milk products when iron is added.[36,37]

Freely Water-Soluble Compounds

Freely water-soluble compounds are the most bioavailable iron compounds, but also the most likely to promote unacceptable color and flavor changes. They are essential in liquid products, and there is often little difference between the compounds with respect to bioavailability, flavor, and organoleptic problems. Ferrous sulfate is the least expen-

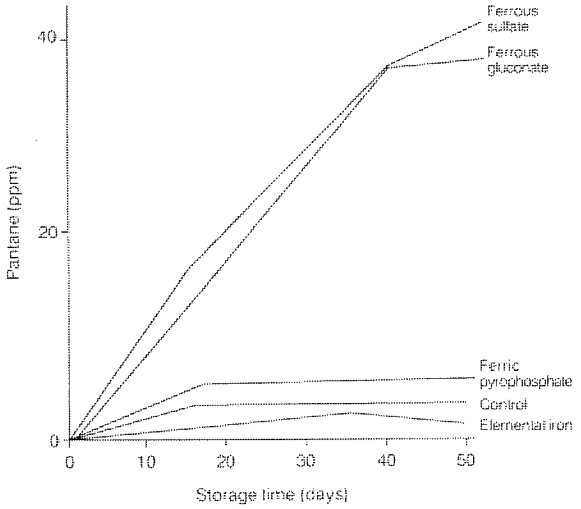

Figure 1. Pentane formation in stored wheat flour fortified with different iron compounds (adapted from reference 27).

sive compound and is widely used to fortify infant formulas and pasta and cereal flour that are stored for only short periods. Other possibilities are ferrous gluconate, ferrous lactate, and ferric ammonium citrate. Although there is no evidence that soluble ferric salts are absorbed to a lesser extent than soluble ferrous salts when iron is in an ionized form,[38] it is possible that ferric iron binds more strongly with inhibitors of absorption such as phytic acid and polyphenols.

Compounds Soluble in Dilute Acid

Recently, several compounds that are poorly soluble in water but readily soluble in dilute acids have been identified. These compounds are ferrous fumarate, ferrous succinate, and ferric saccharate. Their advantage is that they cause far fewer organoleptic problems than freely water-soluble compounds and still readily enter the common iron pool during digestion. They have been suggested for use in infant cereals[35] and chocolate drink powders.[39]

Studies have been conducted in which adult human subjects were fed a chocolate drink or an infant cereal fortified with ^{55}Fe-radiolabeled test compounds or ^{59}Fe-radiolabeled ferrous sulfate.[35,39] The chocolate drink contained 5 mg iron and 25 mg vitamin C per serving, and the infant cereal contained 7.5 mg iron and 35 mg vitamin C per serving. Absolute absorption from the ferrous sulfate control meals varied from 3% to 6%. The absorption from ferrous fumarate and ferrous succinate was at least as good if not better than from ferrous sulfate. Absorption from ferrous fumarate was twice as high as from ferrous sulfate in the chocolate milk drink, and the iron compound may have undergone some reactions during the manufacture of the chocolate drink powder, which included a vacuum drying stage. In the infant cereal, ferrous fumarate was dry-mixed into the product after processing and had an absorption equivalent to the ferrous sulfate. Ferric saccharate had a variable but moderate absorption (RBV 39–74), and ferric pyrophosphate had a variable but low absorption (RBV 20–39). It would seem that these iron compounds are less soluble in gastric juice in the presence of chocolate milk drink than in the presence of infant cereal, because the lowest absorption values were from the chocolate milk drink. Ferric pyrophosphate and ferric saccharate caused no organoleptic problems in either product. In the chocolate drink, ferrous succinate was satisfactory, but ferrous fumarate caused a color loss if the product was made with boiling water. Similarly, ferrous fumarate and ferrous succinate were organoleptically satisfactory when added to simple infant cereals, but color problems occurred in more acid fruit varieties.

Compounds Poorly Soluble in Dilute Acids

Compounds that are poorly soluble in dilute acids include ferric pyrophosphate, ferric orthophosphate, ferric ammonium orthophosphate, and the elemental iron powders made by carbonyl, electrolytic, or reduction techniques.[24,40] They are the most often-used compounds in food fortification and their main advantage is that they cause no organoleptic problems. Their disadvantage is that they have a variable absorption because they do not readily dissolve in gastric juice. Animal studies indicate that current commercial compounds are about half as well absorbed as ferrous sulfate.[35] Human studies, however, have given variable and conflicting results (Table 1[12,24,25]). This is either because the compounds tested had different physiochemical characteristics from the commercial compounds[29,41,42] or because of the influence of different meals on the dissolution of the iron compound in gastric juice. Hallberg et al.,[28] for instance, found that the RBV in humans of the same carbonyl iron powder varied from 5 to 20 and the RBV of ferric ammonium orthophosphate varied from 30 to60[28] simply because of the composition of the meal with which they were fed. When carbonyl iron is consumed without a meal in pharmacologic (100 mg) doses, it is reported to have a relative bioavailability in humans of about 70% that of ferrous sulfate.[43]

It seems probable that the low levels of elemental iron (40 mg/kg) added to wheat flour would have little impact on iron nutrition, but the much higher levels added to commercial infant cereals (200–550 mg/kg) together with vitamin C could contribute substantially to the prevention of iron deficiency anemia.

Encapsulated Iron Compounds

Both ferrous sulfate and ferrous fumarate are available commercially in encapsulated form. Commonly, the coatings are partially hydrogenated oils, such as soybean and cottonseed, or ethyl cellulose. The coating has little influence

on the RBV as measured in rodent assays[25] and can prevent fat oxidation changes during storage of cereals or in infant formulas fortified with the easily oxidizable long-chain polyunsaturated fatty acids. Most coatings are heat labile, however, and at temperatures above 50–70 °C often do not prevent unwanted color reactions. Zinc stearate is the only coating proposed that has a high melting point (122 °C), and its bioavailability in rodent assays was reported to be 70% that of ferrous sulfate.[44]

Protecting and Enhancing the Absorption of Fortification Iron

Many food vehicles for iron fortification contain substances that inhibit iron absorption. Cereals contain phytic acid and occasionally polyphenols, milk contains calcium and casein, and chocolate drinks contain polyphenols. In addition, many diets in developing countries to which fortified salt, sugar, or other condiments are added are often high in phytate and polyphenols from cereal and legume foods. To ensure a level of absorption that is high enough to improve or maintain iron status, it is necessary to prevent the fortification iron from reacting with the absorption inhibitors. This can be accomplished by adding absorption enhancers. The most common enhancer is vitamin C. Alternatives would be bovine hemoglobin and NaFeEDTA where iron is in a protected form.

Vitamin C

Vitamin C can increase the absorption of both native iron and fortification iron severalfold when added to foods. Its effect appears to be related to both its reducing power and its chelating action. It can reduce ferric to ferrous iron and/or maintain ferrous iron in the ferrous state and so prevent or decrease the formation of insoluble complexes with absorption inhibitors or with hydroxide ion in the gut. In addition, it can form soluble complexes with iron at low pH that remain soluble and absorbable at the more alkaline duodenal pH. Thus, Layrisse et al.[45] reported a sixfold increase in iron absorption (1.4% to 7.9%) by adult peasants in Venezuela who consumed 100 g maize containing 2.8 mg iron and 70 mg added vitamin C. Similarly, Cook and Monsen[46] reported that iron absorption in young men fed a liquid formula meal containing 4.1 mg iron increased from 0.8% to 7.1% as vitamin C was increased from 25 to 1000 mg. More recently, Siegenberg et al.[47] reported that the effect of vitamin C on phytate and polyphenols was dose dependant and that as little as 30 mg vitamin C could completely overcome the effect of phytic acid (58 mg phytate phosphorus) in maize bran added to white bread, whereas >50 mg vitamin C overcame the negative effect of meals containing >100 mg polyphenols added as tannic acid.

Vitamin C increases the absorption of all fortification iron compounds to a similar extent.[29] Derman et al.[48] reported that iron absorption by adult women with low iron stores from infant cereal fortified with ferrous sulfate or ferrous ammonium citrate was only about 1% in the absence of vitamin C, but increased fourfold to 10-fold when vitamin C was added. Similarly, Forbes et al.[29] reported that iron absorption by adult men and women consuming a farina and milk meal containing 3 mg iron as ferrous sulfate, ferric orthophosphate, or electrolytic iron was only 1% to 4% in the absence of vitamin C but increased three- to fourfold in its presence.

In a milk-based infant formula fortified with 15 mg iron as ferrous sulfate per liter, iron absorption by infants was only 3% in the absence of vitamin C but increased to 5% with 100 mg vitamin C per liter and to 8% with 200 mg per liter.[49] The poor iron absorption from the product with no added vitamin C was cited as the reason for the relative ineffectiveness of a field trial conducted with this product,[50] but in subsequent field trials with the product containing 100 mg vitamin C per liter, the prevalence of iron deficiency anemia in children 15 months old was only 5.5% compared with 30% in infants receiving a non-iron-fortified formula.[59]

Hemoglobin

Hemoglobin is a form of food iron that is naturally protected from major inhibitors of iron absorption, such as phytic acid and polyphenols. The iron is contained within the porphyrin ring of the heme molecule, which is split from the globin moiety during digestion, and is taken up intact into the mucosal cells.[51,52] The iron is released within the mucosal cell by the action of heme oxygenase[53] and is prevented from reacting with the inhibitory and enhancing ligands within the intestinal lumen. Hemoglobin iron, however, is better absorbed than heme iron without the globin and is further enhanced in the presence of muscle tissue.[54,55] The nature of the mechanism is not fully established, but it seems to be related to protein digestion products preventing the polymerization of heme molecules, thus reducing their absorption.[51]

When used as a food additive, hemoglobin is added in the form of dried red blood cells. Its main advantage is that iron absorption is relatively high and predictable. Absorption varies little with the composition of a meal, and although it varies to some extent with the iron status of the subjects,[56] this variation is far less than with nonheme iron. Monsen et al.[57] estimated that heme iron would be 15–35% absorbed depending on the iron stores; it is thus possible that if hemoglobin-fortified products are not targeted specifically to at-risk groups, tissue iron stores will gradually accumulate in iron-replete subjects. The main disadvantage of hemoglobin iron, however, is the very low iron content (0.34%) and its intense red-brown color. In infant cereal, 5 g dried bovine red blood cells per 100 g rice flour was necessary to provide 14 mg Fe/100 g,[58] making the product dark brown. Iron absorption was 14% in 8-month-old infants, and although the globin protein is lacking in isoleucine, it is high in lysine and is reported to

provide a useful amount of additional protein to a mixed diet.[58] Other disadvantages are the technical difficulties of collecting, drying, and storing animal blood and of obtaining animal blood in countries where meat is not widely consumed, as well as religions beliefs that forbid the consumption of blood.

In Latin American countries where the supply of animal blood is plentiful, two field trials demonstrated the potential usefulness of dried red blood cells as a food fortificant. In the first,[59] extruded rice containing 5% bovine hemoglobin concentrate was fed to infants 4 to 12 months old and their iron status was compared with that of infants fed regular solid foods (vegetables and meat). In the control group at 12 months, the prevalence of iron deficiency anemia was 17% compared with only 6% in infants who consumed more than 30 g fortified cereal per day. In a second study,[21] three 10 g wheat flour cookies containing 6% bovine hemoglobin concentrate were fed as part of the Chilean school lunch program over a period of 3 years. In a survey of 1000 participating children, significantly higher serum ferritin and hemoglobin levels were found in children who consumed the fortified cookies than in those who did not. However, the prevalence of anemia in 10- to 16-year-old schoolchildren was surprisingly low, and in girls the prevalence fell from 1.3% to 0.5%, compared with a fall from 0.8% to 0.4% in boys. The authors concluded that the program would have had a larger impact on iron status in regions where the prevalence of iron deficiency in schoolchildren is higher.

Sodium Iron EDTA

The use of NaFeEDTA as a food additive has recently been reviewed by the International Nutritional Anemia Consultative Group (INACG)[60] and was strongly recommended as the most suitable iron fortificant for use in developing countries. The provisional acceptance of the compound by the Joint FAO/WHO Expert Committee on Food Additives[61] for use in supervised fortification programs in iron-deficient populations has cleared the way for large-scale fortification trials. Other EDTA-containing compounds, i.e., Na_2EDTA and $CaNa_2EDTA$, are widely used in manufactured foods in industrialized countries as protection against metal-induced organoleptic changes. The EDTA molecule forms FeEDTA in the intestinal tract,[60] so that combinations of Na_2EDTA and ferrous sulfate or other iron compounds can also be considered for fortification purposes.

Chemistry. EDTA (ethylene diamine tetraacetic acid) is a hexadentate chelate binding through its four negatively charged carboxylic acid groups and two amine groups. It can combine with virtually every metal in the periodic table. Its effectiveness as a chelate depends on the stability constant between EDTA and the metal. This is influenced by pH and molar ratio, and any metal capable of forming a stronger complex with EDTA will at least partially displace another. Of the nutritionally important metals, Fe^{3+} has the highest stability constant log k of 25.1, followed by copper (Cu) at 18.4, zinc (Zn) at 16.1, Fe^{2+} at 14.6, calcium (Ca) at 10.7, magnesium (Mg) at 8.7, and sodium (Na) at 1.7. The less desirable metals such as mercury (Hg, 20.4), lead (Pb, 17.6), and aluminum (Al, 15.5) and perhaps manganese (Mn, 13.5) also have fairly high stability constants. The situation is somewhat complicated by having an optimum pH for complex formation between 1 and 10. The optimum pH for complex formation between Fe^{3+} and EDTA is pH 1, Cu is 3, Zn is 4, Fe^{2+} is 5, Ca is 7.5, and Mg is 10.[62]

Based on the pH optima, the predicted effect in the intestine of NaFeEDTA and $CaNa_2EDTA$ in food would be as follows. In the stomach, Fe^{3+} from NaFeEDTA would remain firmly bound to EDTA, whereas Ca and Na from $CaNa_2EDTA$ would dissociate and EDTA would bind Fe from the common pool. So even with the addition of $CaNa_2EDTA$, iron EDTA would form in the stomach. In the duodenum, the iron would be released and absorbed[63] and the EDTA would presumably bind in succession to Cu (pH 3), Zn (pH 4), and Fe^{2+} (pH 5), but most of the metals are released for absorption as <5% of the metal–EDTA complexes are absorbed (<1% FeEDTA)[64] and excreted directly in the urine. More than 95% of the EDTA molecule is excreted in the stool. Theoretically, in the ileum and colon, it could bind to Ca, which has a pH optimum of 7.5 for complex formation. Mg, with a low stability constant and a high pH optimum of 10.5, probably would not react.

Absorption of Iron from NaFeEDTA. The major advantage of NaFeEDTA over other iron fortification compounds is that it prevents iron from binding with the phytic acid present in many cereal and legume grains. Thus, in cereal foods or meals containing a considerable quantity of phytic acid, the absorption of iron from NaFeEDTA is two- to threefold that from ferrous sulfate. With less inhibitory foods, such as potato, there is little difference between the iron absorption from the two iron compounds. With neutral foods, such as sugar cane syrup, consumed on their own, iron absorption when fortified with NaFeEDTA was only 30% of that from ferrous sulfate (for detailed review see reference 60).

In a way similar to vitamin C, Na_2EDTA could be considered an absorption enhancer. It has the added advantage of being stable during processing and storage. It must, however, be added at an equivalent or slightly lower molar ratio to iron in the meal. El-Guindi et al.[65] added equimolar quantities of ferrous sulfate and Na_2EDTA to Egyptian *baladi* bread and increased iron absorption from 2.1% to 5.3%. Earlier work suggested that increasing the ratio of Na_2EDTA to iron is associated with a progressive reduction in iron absorption.[66] MacPhail and Bothwell[60] recently reported that adding Na_2EDTA to a ferrous sulfate–fortified rice meal significantly increased absorption at EDTA-to-iron ratios of 1:4 to 1:1, with a maximum absorption at

1:2. EDTA-to-iron ratios of 2:1 to 4:1 did not significantly increase or decrease iron absorption.

Possible Reactions of EDTA with Other Dietary Minerals. Considering the possible impact of EDTA from NaFeEDTA (10 mg iron per day) on the nutritional status of other minerals assumed to be in the diet at levels equivalent to their RDAs, it can be calculated that on a molar ratio basis there are 50 times more magnesium and 80 times more calcium than EDTA, so there would be no likely impact of EDTA on magnesium or calcium metabolism. With copper and zinc, however, there could be a possible effect, since on a molar basis there are eight times more EDTA than copper and equivalent amounts of EDTA and zinc.

We have investigated this effect in both rodents and adult women. In rodents, increasing levels of EDTA in the diet increased zinc absorption and, to a lesser extent, also increased copper absorption but had no effect on calcium absorption.[67] In adult women fed iron-fortified bread rolls, zinc absorption was increased from 20% with ferrous sulfate to 34% with NaFeEDTA, although there was no effect on calcium absorption. Urinary zinc excretion was also increased from 0.3% to 0.6%, but this had little or no effect on overall zinc metabolism.[68] The EDTA molecule from added NaFeEDTA can therefore increase both iron and zinc absorption from meals containing phytic acid. It might also increase the absorption of copper, as well as the potentially toxic elements Pb, Hg, Al, and Mn. However, it would be expected to have no effect on calcium and magnesium absorption.

Intervention Studies. Three intervention studies have been made with NaFeEDTA by Garby and Areekul[69] in Thailand, Viteri et al.[70,71] in Guatemala, and Ballot et al.[72] in South Africa. All were controlled studies, but only the South African study was double blinded. The number of subjects varied from approximately 600 to 17,000 and the study time from 12 to 32 months. None of the food vehicles—fish sauce, sugar, curry powder—contained phytic acid. The amounts of iron provided per day were 4.3 mg in sugar, 7.7 mg in curry powder, and 10–15 mg in fish sauce. All showed a positive effect on iron status. In the fish sauce study, packed cell volume increased in men, women, and children. In the sugar study, even with a fairly low level of fortification and a relatively modest compliance, there was an increase in serum ferritin (iron stores) in all subjects receiving the fortified product but not in subjects receiving the unfortified product. In the curry powder study, there was an increase in red cell hemoglobin levels and serum ferritin in all subjects, and anemia in women fell dramatically from 22% to 5%.

Organoleptic Considerations. Iron combined in NaFeEDTA causes fewer organoleptic problems than other water-soluble iron compounds. It can, however, cause unwanted color changes. We have found it to be unsuitable for the fortification of chocolate drink powders and infant cereals containing banana and other fruits. Viteri et al.[71] reported that NaFeEDTA-fortified sugar is slightly yellow in color and, when added to tea, turned the tea black. Similarly, when added to corn starch puddings and gruels, it turned them a pinkish-violet color.

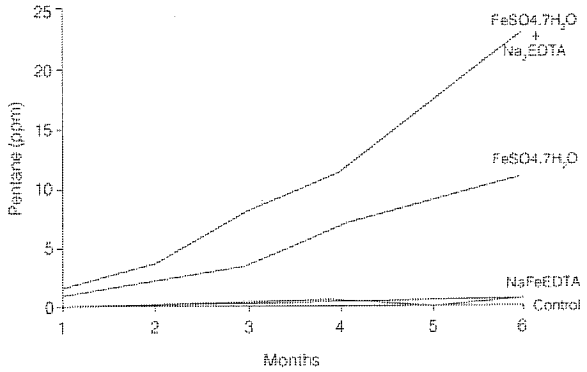

Figure 2. Pentane formation in stored wheat flour fortified with NaFeEDTA.

NaFeEDTA does have an advantage, however, when added to stored cereals, because unlike ferrous sulfate, it does not provoke the fat oxidation reactions that lead to rancid, oxidized products. We stored (unpublished results) dry white wheat flour mixed with NaFeEDTA, ferrous sulfate, or ferrous sulfate plus equimolar Na_2EDTA (15 mg Fe/100 g) in closed aluminum cans as described by Hurrell et al.[35] Fat oxidation was quantified by measuring the accumulation of pentane in the headspace. The results (Figure 2) show that stored wheat flour underwent little or no fat oxidation during 6 months storage at 37 °C when unfortified or fortified with NaFeEDTA. In contrast, when the flour was fortified with $FeSO_4\ 7H_2O$ (hepta hydrate), or $FeSO_4\ 7H_2O$ plus Na_2EDTA, lipids in the wheat flour were progressively oxidized during the storage periods and progressively more pentane accumulated in the headspace.

Regulatory Issues and the Current Use of EDTA in Foods. The Joint FAO/WHO Expert Committee on Food Additives (JECFA)[73] permitted the use of $CaNa_2EDTA$ and Na_2EDTA up to 2.5 mg/kg body weight/day with a maximum acceptable daily intake (ADI) set at 150 mg/person/day. The ADI was extrapolated from the rodent study by Oser et al.[74] as the highest no-effect level (250 mg/kg), applying a safety factor of 100. Unfortunately, this study did not include higher levels of EDTA. These compounds are now permitted by local food and drug authorities for use in many countries in Asia, Africa, the Middle East, Europe, and America as a sequestering agent for metals to prevent flavor changes, rancidity, discoloration, turbidity, and texture loss. They are most often added to foods such as mayonnaise, canned vegetables (peas, beans, potatoes), canned fish and shell fish, carbonated beverages, beer, and margarine. In the United States, they are permitted in 34 different foods at levels varying from 33 to 800 mg/kg (Table 2), although the estimated daily intake is only 25

Table 2. Examples of Approved $CaNa_2EDTA$ Use in Foods in the United States

Food Products	Purpose	Amount Permitted (mg/kg)
Lima beans, canned	Retain color	310
Pinto beans, dried	Retain color	800
Cabbage, pickled cucumber	Retain color, flavor texture	200
Carbonated beverages	Retain flavor	33
Crabmeat, clams, shrimps	Retain color	250–340
Egg products	Preservative	200
Margarine	Retain color	75
Mayonnaise	Retain color	75
Mushrooms, canned	Retain color	200
Potatoes, canned	Retain color	110
Sandwich spread	Preservative	200

mg/person/day,[60] 10 times less than the ADI.

Although other regions, such as Malaysia and the Philippines, also allow EDTA in a wide range of foods, the European Union takes a more restrictive view and only allows addition to canned crab, canned shrimp, pickles, canned mushroom, glacé cherries, and sauces. EDTA compounds are currently not allowed in foods consumed by infants and young children.

Present Status of NaFeEDTA. Although NaFeEDTA would appear, at present, to be the most appropriate iron fortificant for use in developing countries, it is still about six times more expensive than ferrous sulfate. However, it is two- to threefold better absorbed than ferrous sulfate, and relatively expensive vitamin C does not need to be added as an absorption enhancer. Additional savings can be made in the packaging material, because less sophisticated packaging can be used for a NaFeEDTA-fortified food than for one fortified with ferrous sulfate (or other iron salts) and vitamin C. The better packaging material must be designed to protect vitamin C from degradation during storage.

However, before general use of NaFeEDTA can be recommended, more systematic studies are necessary to ascertain potential organoleptic problems in a variety of foods. Additionally, its influence on the absorption of the potentially toxic metals (Pb, Hg, Al, Mn) must be investigated and the physiologic importance of any demonstrated influence must be ascertained.

Food Vehicles for Iron Fortification

Cereal Products

Cereal flours are currently the most frequently used vehicles for iron fortification that reach the entire population. The amount of iron added is usually relatively low because it is added only to restore the iron level in milled flour to that of the whole grain. With true fortification, a higher amount than is usually present would be added. Wheat flour enrichment is mandatory in many countries, and the native level in 70% extraction flour (11–12 mg/kg) is enriched up to 44 mg/kg, which is the approximate content of whole-wheat grains. This is the situation in the United States. Other countries add even lower amounts of iron. In Denmark, the enrichment level is 30 mg/kg and in the United Kingdom it is 16.5 mg/kg, as the iron content in white flour is restored to that of 80% extraction flour.

In the United States, corn (maize) meal, corn grits, and pasta products also have federal standards for voluntary iron enrichment, and these commodities are mostly enriched by manufacturers similarly to other baked goods such as crackers, rolls, cookies, and doughnuts but to a lesser extent.[75] The contribution of fortified iron to iron intake is highest in the United States, where it accounts for 20–25% of total iron intake.[76,77] The contribution of fortified iron to iron intake in the United Kingdom is much lower, around 6%.[19]

Technology also exists for fortifying whole grains such as rice. This can be done by coating, infusing, or by using extruded grain analogues. The fortified grains are then mixed 1:100 or 1:200 with the normal grains. Hunnell et al.[78] described a sophisticated method of preparing fortified rice grains by first infusing B vitamins and then adding iron, calcium, and vitamin E in separate layers of coating material. The cost of these procedures together with the difficulty of completely masking the fortified grains is the main reason why no successful programs have been implemented in developing countries. Although iron fortification of rice is mandatory in the Philippines, it has never been enforced.[11]

Other commonly fortified foods are breakfast cereals and infant cereals. In industrialized countries, breakfast cereals can potentially provide a significant amount of iron, particularly to children and adolescents. In the United Kingdom, for instance, they can provide up to 15% of total iron intake in 11–12-year-olds.[79] The contribution of fortified iron from infant cereals is potentially much greater because they often provide the major source of iron at a critical time in a child's growth and brain development.

There are two major disadvantages to using cereal

products as vehicles for iron fortification. First, they contain high levels of phytic acid, a potential inhibitor of iron absorption[6]—up to 1% in whole grains and about 100 mg/100 g in high-extraction flours. Second, they are extremely sensitive to fat oxidation during storage when highly bioavailable iron compounds such as ferrous sulfate are added.[35] For organoleptic reasons, cereal flours such as wheat and maize are usually fortified with poorly absorbed elemental iron powders, and rice with ferric orthophosphate or ferric pyrophosphate.[78] Only bread, wheat flour stored for less than 3 months, and pasta products, because of their low moisture content, can be fortified with the more highly available ferrous sulfate.[80] However, even with these foods, iron absorption will be inhibited by the presence of phytic acid unless an absorption enhancer is present. This is rarely the case, although NaFeEDTA would appear to be ideally suited to the fortification of cereal flours and perhaps even pasta products. The usefulness of the fortification of these cereal foods can therefore be questioned, because rather low levels of poorly absorbed iron compounds are added without absorption enhancers to products containing phytic acid.

Breakfast cereals are similarly fortified with reduced elemental iron,[80] and in the absence of vitamin C, the usefulness of this fortification is also doubtful. Infant cereals, by contrast, are fortified with much higher levels of iron (200–500 mg/kg) in the presence of large amounts of vitamin C. More bioavailable iron compounds such as ferrous fumarate are also often used,[35] and even with the electrolytic form of elemental iron, the efficiency of infant cereals to provide a nutritionally useful source of iron has been demonstrated.[30]

Salt

Iodine-fortified salt has successfully eradicated iodine deficiency in many countries,[81] so salt would also seem a highly suitable vehicle for iron fortification. However, iron fortification of salt poses many technical problems, and for developing countries, an efficient production and distribution system must also exist.

Almost all of the development work for the fortification of salt with iron has been conducted in India.[82-85] Color changes during storage have been the main problem, because salt in India is relatively crude and contains up to 4% moisture. All soluble iron compounds and vitamin C caused unacceptable color changes. Fortification was possible only with insoluble iron compounds, and ferric orthophosphate was recommended at 1 mg iron per gram salt so as to provide about 15 mg extra iron per day. When $NaHSO_4$ was added as an absorption promoter,[34] absorption was reported to be 80% that of ferrous sulfate. A small-scale fortification trial in which the fortified salt was included in a school feeding program demonstrated an improvement in iron status.[84]

Salt that contains fewer impurities would undoubtedly be easier to fortify, but the extra cost to the consumer is always a major consideration in developing countries. In addition, there is always the possibility that the iron-fortified salt will cause unacceptable color reactions if added to vegetables in a meal. This was one of the explanations offered for the failure of a salt fortification program in the Seychelles and Mauritius in the early 1960s.[86] The other reasons were the relatively poor bioavailability of the ferric pyrophosphate used and the fact that it separated from the salt and sank to the bottom of the salt barrels.

Sugar

Sugar is an alternative vehicle for iron fortification in regions of the world where it is produced, such as the Caribbean and Central America, but in other developing countries refined sugar consumption is more common in the middle and upper socioeconomic segments of the population.[11] Iron from fortified sugar would be expected to be well absorbed if consumed with citrus drinks but poorly absorbed from coffee and tea owing to phenolic compounds or, if added to cereal products, owing to phytate.

As with salt, the main technical problem is to select a bioavailable iron compound that does not cause unwanted color changes in less pure sugar products. In Guatemala, this was overcome by adding NaFeEDTA.[71] Commercial white cane sugar would appear easier to fortify, and Disler et al.[32] reported the successful addition of several different ferric and ferrous compounds (100–200 mg iron/kg) together with vitamin C. There were, however, unacceptable color reactions when added to coffee and tea[32] or to certain maize products.[71] A successful fortification trial was reported in Guatemala, where NaFeEDTA added to sugar at 13 mg iron/kg to provide an extra 4 mg iron/day per person increased iron stores in all population groups receiving the fortified product.[71]

Milk

Infant formulas are usually milk based with added vegetable oils, minerals, and vitamins. Iron is almost always added as ferrous sulfate from 5 to 12 mg per liter,[87] and its absorption can be improved considerably by the addition of 100–200 mg vitamin C per liter.[49] The relatively low iron bioavailability from milk products can be assumed to be due to the presence of two inhibitory factors, calcium[88] and the milk protein casein.[89] In a series of fortification trials in Chile in which iron-fortified formulas were fed to infants, the improvement of iron status was only modest in the absence of vitamin C but improved considerably when it was added to formula.[20] The widespread consumption of iron-fortified (and vitamin C-fortified) formulas by infants in the United States is regarded as the reason for the dramatic fall in the prevalence of anemia over the last 30 years.[16]

Whole milk could also be considered as a vehicle for iron fortification, but because of the presence of calcium and casein, an absorption enhancer should be added to

improve absorption. Unfortunately, it is difficult to add vitamin C to fluid milk and it has been reported to degrade rapidly to diketogluconic acid leading to changes in flavor.[90] Many soluble iron compounds rapidly produce off-flavors when added to milk, owing to the promotion of lipolytic rancidity, oxidative rancidity by the oxidation of free fatty acids, and the partial or complete loss of vitamins A, C, and ß-carotene.[91]

After evaluation of a series of compounds, the addition of ferric ammonium citrate has been proposed for liquid milk[36,92] and for skim milk, skim milk concentrate, and dry milk powder.[93] The addition of NaFeEDTA would appear to be an interesting alternative, but it has not been evaluated extensively for organoleptic properties in milk. The usefulness of milk as a vehicle for iron fortification has been demonstrated in a Mexican school feeding program.[93] The hemoglobin level of children fed 200 mL milk containing 20 mg iron as ferrous chloride improved by 1 g/dL in 3 months. This study demonstrated that with high levels of added iron, the addition of vitamin C was not essential. As with iron-fortified sugar, when iron-fortified milk is added to tea, coffee, or cocoa, the beverages undergo unacceptable color changes.

Iron-fortified milk-based chocolate drinks are also food products that can be usefully targeted to children and adolescents. A variety of products are commercially available, although the phenolic compounds present in cocoa powder readily undergo color changes with soluble iron[33] and also bind iron in the gut and inhibit its absorption. Compounds such as ferrous fumarate, ferrous succinate, ferric saccharate, and ferric pyrophosphate have shown acceptable organoleptic properties,[39] with fumarate showing the highest absorption. The addition of vitamin C would presumably be necessary to overcome the inhibitory factors in the cocoa and milk.

Condiments

Condiments that are traditionally used in developing countries, such as monosodium glutamate, fish sauce, curry powder, and bouillon cubes, could be useful fortification vehicles. Monosodium glutamate is widely used as a flavor enhancer in Asia and has been successfully fortified with ferric orthophosphate and ferrous sulfate encapsulated in zinc stearate.[44] The latter compound had 70% of the relative bioavailability of ferrous sulfate in rodents and the capsule had a melting point of 122 °C. Pilot fortification trials with iron-fortified fish sauce[69] or curry powder,[72] both fortified with NaFeEDTA, resulted in significant improvement in iron status in the population consuming the fortified products. The success of fortified condiments presumably depends both on the absence of adverse color reactions and on the addition of an absorption enhancer, such as EDTA.

Coffee

In some populations coffee is consumed by most adults as well as some children, and it is technically and economically feasible to fortify coffee with iron. Johnson and Evans[65] reported the use of ferrous fumarate in roasted and ground coffee, in which one cup (200 mL) provided 1 mg added iron. The addition of iron to soluble coffee is also relatively easy; Klug et al.[96] reported that the addition of a range of soluble ferrous and ferric compounds was possible. Flavor and color changes, however, are a potential problem, and coffee, like tea and cocoa, contains phenolic compounds that strongly inhibit iron absorption.[97]

Conclusion

One strategy to overcome the high prevalence of iron deficiency anemia in developing countries is to fortify various food products with iron. There are several options with respect to the iron compound used and the food product to be fortified. Various factors, including cost effectiveness of the fortification in raising absorbable iron intake in the targeted population, the palatability of the fortified food, and the etiology of iron deficiency must be considered before initiating a fortification program. As most iron-fortified foods contain potential absorption inhibitors, it is essential to protect the fortification iron so as to ensure adequate absorption. This can be achieved easily in the food industry by adding vitamin C, although EDTA and, possibly, hemoglobin would seem better options for developing countries.

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EUROPEAN COMMISSION
Joint Research Centre

Institute for Health and Consumer Protection
European Chemicals Bureau
I-21020 Ispra (VA) Italy

EDETIC ACID (EDTA)

CAS No: 60-00-4

EINECS No: 200-449-4

Summary Risk Assessment Report

2004

Special Publication I.04.279

EDETIC ACID (EDTA)

CAS No: 60-00-4

EINECS No: 200-449-4

SUMMARY RISK ASSESSMENT REPORT

Final report, 2004

Germany

The risk assessment of edetic acid (EDTA) has been prepared by Germany on behalf of the European Union.

Contact point:

Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA)
Anmeldestelle Chemikaliengesetz
(Federal Institute for Occupational Safety and Health-Notification Unit)
Friedrich-Henkel-Weg 1-25
44149 Dortmund

Fax: +49 (231) 9071 679
e-mail: chemg@baua.bund.de

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PREFACE

This report provides a summary, with conclusions, of the risk assessment report of the substance edetic acid (EDTA) that has been prepared by Germany in the context of Council Regulation (EEC) No. 793/93 on the evaluation and control of existing substances.

For detailed information on the risk assessment principles and procedures followed, the underlying data and the literature references the reader is referred to the comprehensive Final Risk Assessment Report (Final RAR) that can be obtained from the European Chemicals Bureau[1]. The Final RAR should be used for citation purposes rather than this present Summary Report.

[1] European Chemicals Bureau – Existing Chemicals – http://ecb.jrc.it

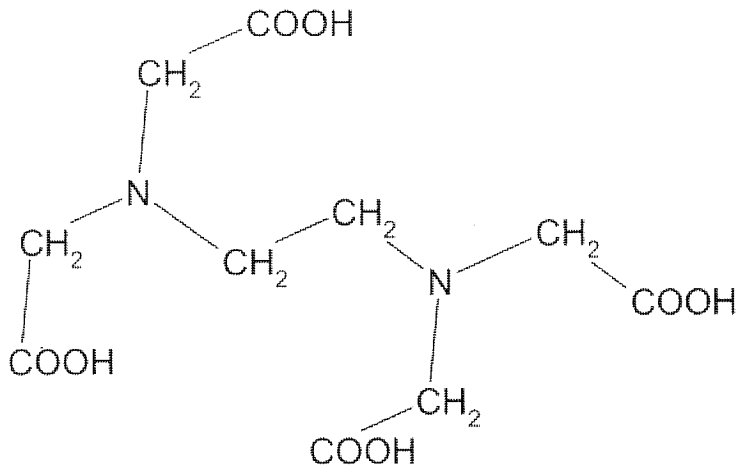
CONTENTS

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No:	60-00-4
EINECS No:	200-449-4
IUPAC Name:	{[2-(Bis-carboxymethyl-amino)-ethyl]-carboxymethyl-amino}acetic acid
Synonyms:	ethylenediaminetetraacetic acid; ethylenedinitrilotetraacetic acid; N,N'-1,2-ethanediylbis[N-(carboxymethyl)glycine]; edetic acid; H_4EDTA; EDTA
CA Index name:	Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)-
Empirical formula:	$C_{10}H_{16}N_2O_8$
Molecular formula:	292.3 g/mol
Molecular weight:	292.3 g/mol
Structural formula:	



1.2 PURITY/IMPURITIES, ADDITIVES

Purity:	98-100% w/w
Impurity:	< 0.3 trisodium nitrilotriacetate < 0.3 ethylenediaminetriacetate < 0.3% w/w nitrilotriacetic acid < 1% water
Additives:	none

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 1.1 Physico-chemical properties

Parameter	Value
Physical state	at 20°C, 1,013 hPa: colourless crystals
Melting point	n.a. (decomposition above 150°C)
Boiling point	not applicable [1]
Relative density	0.86 at 20°C
Vapour pressure	not determined [2]
Surface tension	not determined [3]
Water solubility	0.4 g/l at 20°C
log P_{ow}	-5.01 (calculated according Hansch and Leo) [4] -3.34 (calculated according Rekker)
Flash point	not determined because substance is a solid
Flammability	not flammable [5]
Ignition temperature	no self ignition up to the decomposition at 150°C (VDI 2263)
Explosive properties	not explosive [6]
Oxidising properties	no oxidising properties [6]
Henry's law constant	1.10^{-20} Pa.m³/mol [7]

1) A determination of the boiling point is scientifically not meaningful because the substance decomposes above 150°C
2) The vapour pressure is estimated to be very low for particularly ionic substances. Therefore a determination was not conducted.
3) The surface tension was not determined because of structural reasons.
4) This value is used in the following risk assessment.
5) In a preliminary test the ignition level was determined to be 1. Therefore the substance is not flammable.
6) No test was conducted because of structural reasons.
7) As no value for the vapour pressure is known, a Henry's law constant can not be calculated from vapour pressure and water solubility. So a fictitious low value is used for the risk assessment.

1.4 CLASSIFICATION

Classification according to Annex I

Class of danger: none
R-Phrase: none

The classification of H_4EDTA is not included in Annex I to Directive 67/548/EEC.

Proposed classification

At the meeting of 17-19 November 2003 the EU classification and labelling working group (Human Health) agreed upon the following classification for EDTA:

Xi; R36

Labelling

Xi
R: 36
S: (2-)26

Xi	Irritant
R36	Irritating to eyes
S2	Keep out of the reach of children
S26	In case of contact with eyes rinse immediately with plenty of water and seek medical advice

2 GENERAL INFORMATION ON EXPOSURE

EDTA is mainly produced and used as acid (H_4EDTA) and as sodium salt (Na_4EDTA). In lower amounts, other salts or metal complexes are produced or used. The environmental exposure from the different uses of all EDTA species is overlapping. Thus, for the environmental risk assessment (Sections 2 and 3) all production and use volumes are given as H_4EDTA equivalents.

Na_4EDTA is synthesised preferably by cyanomethylation of ethylene diamine with sodium cyanide and formaldehyde. Alternatively, a two-step reaction is in practise: first hydrogen cyanide reacts with formaldehyde to ethylene dinitrilo tetraacetonitrile, which is in the second step hydrolysed with sodium hydroxide to Na_4EDTA. From its salt, H_4EDTA is produced by acidification with sulphuric acid and precipitation from aqueous solution.

In the European Union, EDTA is produced or imported at 7 sites. During production, releases occur via wastewater into surface waters. According to the data submitted by the producers, the total yearly releases into the hydrosphere amount to 266 tonnes/annum. The total yearly releases as dust into the atmosphere are 11 tonnes/annum.

EDTA is used as a complexing agent in many industrial branches. The substance is sold either directly from the producers to the consumers or via distributors. The European application volumes (referred as H_4EDTA) were in 1999:

Table 2.1 European use pattern of EDTA

Use	Marketed amount [t/a]
Household detergents	2,619 (7.6%)
Industrial and institutional detergents	10,685 (31%)
Photochemicals	4,191 (12%)
Textiles	639 (1.8%)
Pulp and paper	4,002 (12%)
Metal plating	470 (1.4%)
Agriculture	5,821 (17%)
Cosmetic	756 (2.2%)
Rubber processing	469 (1.4%)
Oil production	358 (1.0%)
Exports	1,143 (3.3%)
Others	3,393 (9.8%)
Total	34,546

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

Environmental releases

During the use as complexing agent, the major amount of the applied EDTA is emitted into the wastewater. The emission situation in the individual industry branches is presented in Section 3.1.2.2.

When H_4EDTA and Na_4EDTA are emitted during production, use etc., the same ionic species are formed in the environment, independent to the originally used compound (acid or a salt). Therefore, in the environmental exposure assessment the emissions from both H_4EDTA and Na_4EDTA uses have to be added. In order to obtain comparable values, H_4EDTA equivalents are calculated for emissions of the Na-salt or metal complexes, and the environmental risk assessment is performed on this basis. Also in the literature all figures are generally related to H_4EDTA; when the species is not stated they will be taken as H_4EDTA.

Environmental fate

Degradation

The results of standard biodegradation tests can not be used for the exposure assessment. It was demonstrated by laboratory tests that biodegradation of EDTA is strongly dependent on the complexed metal. Metal-EDTA complexes with a thermodynamic stability constant below 10^{12}, like Ca, Mg and Mn, were degraded under special conditions, while chelates with stability constants above 10^{12}, such as Cu and Fe, are recalcitrant.

In biological treatment plants, EDTA can be removed when a number of specific conditions are present: a relatively high hydraulic and sludge retention time, an alkaline pH value of the wastewater, and when EDTA is not complexed with heavy metal ions. Monitoring data indicate that these conditions are fulfilled in some treatment plants of paper mills and beverage industry, where removal rates up to 95% were determined. In contrast, in municipal treatment plants EDTA is generally not removed. In the exposure scenarios for paper mills and beverage industry, 2 scenarios were calculated. In the first scenario no removal is assumed reflecting a worst-case scenario, and a removal of 90% reflecting the best available techniques. In all other scenarios no removal is assumed.

In sediment and soils, EDTA is aerobically degraded with estimated half-lifes of 300 days.

Fe (III) EDTA was found to be photolytically degraded in aqueous solution. As a relevant part ($^1/_3$ to $^2/_3$ of total EDTA) is released as iron complex, this mechanism is probably predominant in environmental EDTA degradation. For the regional exposure scenario, it is assumed that 50% of the total EDTA releases are photolytically degraded with a half-life of 20 days.

The pathway of both biodegradation and photolysis is identical: carboxymethyl moieties are subsequently cleaved, leading to ethylenediaminetriacetic acid (ED3A) as the first reaction product. ED3A is either further degraded or undergoes intramolecular cyclisation leading to ketopiperazinediacetate (KPDA). The sum of ED3A and KPDA was detected in surface waters in concentrations up to 16 µg/l. KPDA was found to be readily biodegradable failing

the 10-days window. Due to the degradation properties, accumulation of the EDTA metabolites is not expected.

Distribution

EDTA and its metal complexes are highly soluble in water. Because of the ionic properties, volatilisation from aqueous solution will not occur.

The adsorption onto sediments is low: For 3 metal complexes, partitioning coefficients between 3 and 113 l/kg were determined. For the exposure calculation, values for Kpsoil, Kp_{sed} and Kp_{susp} of 75 l/kg are used.

Accumulation

Bioaccumulation of EDTA and its metal complexes is low: for the exposure assessment a BCF of 1.8 l/kg was used.

3.1.2 Aquatic compartment (incl. sediment)

3.1.2.1 Production

Exposure scenarios were calculated based on site-specific data of 7 European EDTA producers. The calculated PEClocal values are in the range of 0.095 and maximum 1 mg/l.

3.1.2.2 Use

Releases into household sewage

EDTA is an ingredient of household detergents, cosmetics, pharmaceuticals and food. The main function is the complexation of trace metals. It was assumed that the total application volume (3,669 tonnes/annum) is released via the household sewage without elimination in treatment plants. The resulting PEClocal is 0.195 mg/l.

Industrial detergents

EDTA prevents the precipitation of calcium, magnesium and heavy metals which can cause sedimentation and incrustation in containers, pipes, nozzles and on planes to be cleaned. In alkaline degreasing fleets, phosphates are stabilised and the flocculation of calcium soap is prevented, furthermore the cleaning effect is intensified and tarnishing of metal surfaces is prevented.

The total European market volume was 10,685 tonnes in 1999. There are a large number of use areas within the industrial and institutional detergents (I&I) market. The products formulated to incorporate EDTA described within these functions are distributed to a large number of outlets thus resulting in disparate entry of EDTA to the aquatic environment, mainly via municipal effluent treatment systems. A high level of usage is the dairy and beverage industry, with 50% of the total reported tonnage. The majority of users within the dairy and beverage industry use less than 1 tonne/annum, but three large sites with consumption up to 30 tonnes/annum are known. For the exposure estimation, 3 alternative scenarios are regarded:

- The first scenario should reflect the situation for the majority of the sites. Assuming that the total amount is emitted into the municipal wastewater, a PEClocal of 0.64 mg/l is obtained.
- The second exposure scenario describes the exposure from dairy and beverage sites with a consumption of 10 tonnes/annum. As a worst-case approach, no effective removal in treatment plants is assumed. A PEClocal of 2.6 mg/l is calculated.
- For the third scenario, the same EDTA consumption as for the second (10 tonnes/annum) is regarded, but 90% elimination in a long-termed aerated biological treatment plant (LAS) reflecting the best available techniques is assumed. The resulting PEClocal is 0.35 mg/l.

Photochemicals

In the photoindustry Fe(III)NH_4EDTA is mainly applied in the bleachfix process which is a combination of bleaching (oxidation of the metallic silver) and fixing (removing of silver ions by complexation). The exposure scenario represents a large photofinisher, for which a PEClocal of 0.57 mg/l is calculated.

Wastes from photoindustry are collected by disposal companies. Bath residues are either incinerated or evaporated and deposited, and uses in the cement industry for nitrogen oxide removal from fumes are known. Some monitoring data are available which indicate that at disposal sites EDTA is released into the wastewater. Based on TGD default values, a PEClocal of 2.4 mg/l is calculated.

Textile industry

EDTA is used in textile finishing to support processes like cross linking of cellulose molecules (to produce easy care fabrics) and oxidative bleaching and to prevent catalytical damages of the fibres. The exposure scenario for large textile finishing sites results in a PEClocal of 2.0 mg/l.

Pulp and paper

Bleaching agents are applied by paper mills to remove remaining lignin from the cellulose fibres and to improve the brightness. If hydrogen peroxide is used as bleaching agent, heavy metals like manganese would decompose the peroxide; therefore they have to be chelated. EDTA is not fixed onto the paper; therefore the total use amount is emitted into the sewage.

The exposure estimation is mainly based on site-specific data for 11 Swedish paper mills covering about 75% of the European paper production. 2 scenarios are calculated.

- Monitoring data for mills effluents are reported which demonstrate that EDTA is partially removed in long-termed aerated biological treatment plants. A removal factor of 90% is chosen for those sites reflecting the best available techniques, resulting in a PEClocal of 0.5 mg/l.
- As only a part of the treatment plants are run under favourite EDTA degrading conditions (alkaline pH, long sewage and sludge residence time), at other plants no elimination is assumed as a worst-case approach. This scenario leads to a PEClocal of 4.1 mg/l.

Monitoring data from Swedish and Finnish sites (representing the majority of the European market) reveal that a PEClocal of 4.1 mg/l resulted from the worst-case scenario is not reached for any site. Therefore, this value is not used in the risk characterisation. Instead, the

highest Clocal (2.5 mg/l) derived from monitoring data (leading to a PEClocal of 2.6 mg/l) is considered as the worst case.

Metal Plating

EDTA is used for the production of printed circuit boards. EDTA is mainly used in electroless copper plating, when copper is deposited on the board by catalytic reduction of complexed copper compounds. The exposure scenario, based on the average EDTA consumption of one site and TGD default values for the dilution model, results in a PEClocal of 12 mg/l.

Water treatment

EDTA is used to clean scale deposits from internal boiler surfaces and as additive to incoming boiler feedwater to prevent the formation of calcium and magnesium scales. It is assumed that the 215 tonnes EDTA yearly used in Europe for this purpose are widespread and will not lead to a high local exposure. Thus for this use a PEC is not calculated.

Polymer and rubber production

EDTA is used in the production of Styrene Butadiene Elastomers (SBR) which is mainly manufactured by emulsion polymerisation. EDTA is a sequestering agent for Fe(II)/Fe(III) ions in the initiator system. Based on the TGD default model, a PEClocal of 1.7 mg/l is calculated.

Oil production

EDTA is used for well cleaning processes at oil platforms. In the cleaning process about 1 tonne of EDTA is used during a 24-hour period, leading to intermittent releases. According to the CHARM (Chemical Hazard and Risk Management) model, a dilution factor of 1,000 is reached at a distance of 500 m from the platform leading to a PEClocal of 0.63 mg/l.

Fuel gas cleaning

EDTA is an ingredient for fume desulfuration at coal power plants and waste incineration plants according to the Wellmann-Lord-process. The EDTA containing solution is run in a circle, and a partial stream is incinerated, thus there are no emissions into the wastewater.

Disposal

From different uses, EDTA containing wastes come up from the industrial processes. From the photoindustry it is known that minilabs are (partially) run waste-water-free, the wastes being collected by waste disposal companies. Furthermore, bath residues from photofinishers and probably from other branches like textile finishing or circuit board production are collected.

There is some monitoring data available indicating that EDTA is released into the wastewater at disposal sites. In the frame of the present risk assessment, it was not possible to gain more information about environmental releases for this life-cycle step. Therefore, the results from photochemicals recovery are used for the risk characterisation (PEClocal = 2.4 mg/l).

Sediments

Because of the low partitioning coefficients, no accumulation in sediments will take place. Thus an assessment of this sub-compartment is not necessary.

Monitoring in municipal treatment plants

During an extended monitoring program in German municipal WWTP effluents, in 1994-1995 the EDTA concentration exceeded 600 µg/l in 5 of 55 sampled plants. In Switzerland, the EDTA concentrations in wastewater are generally in the range between 10 to 500 µg/l, with maximum loads between 1,000 to 5,000 µg/l.

Monitoring in surface waters

During an extended monitoring program, EDTA was measured in German surface waters. From 1993 to 1995, the substance was sampled at 143 locations at 73 rivers and creeks, with 1 to 24 samples per year at each location. The EDTA concentration exceeded 500 µg/l at 2 sampling sites. The highest detected concentration in a creek was 2,000 µg EDTA/l, however this was a singular result.

In 1994, the concentrations were 4.1-17.6 (means 8.69) µg/l in the Rhine at Lobith and 3.5-11.4 (means 7.7) µg/l in the Ijsselmeer at Andijk. At Lobith, the average concentrations were 7.7 µg/l in 1995, 10.9 µg/l in 1996, and 7.0 µg/l in 1997.

In the Lake Constance near Überlingen, the yearly averaged EDTA concentration was 4.8 µg/l in 1989. The value decreased to 2.5 µg/l in 1994.

In Swiss rivers, the EDTA concentrations are generally below 20 µg/l. In the river Glatt, maximum concentrations of about 200 µg/l were measured.

In the river Odiel near Huelva (Spain) EDTA was measured at two sites. The first sampling point is near several industrial emission sources, the EDTA concentration was 2.46 mg/l. The second site near the river mouth is influenced by sea water, the EDTA concentration was 0.599 mg/l.

3.1.2.3 EDTA metal complexes in the hydrosphere

The most important property of EDTA is the formation of water soluble complexes with multivalent metal ions. Metal ions are ubiquitously present in wastewater and surface waters, thus the agent is always completely complexed in both media. Because of the high complex formation constants, heavy metal ions are bound preferredly. In the thermodynamic equilibrium, the most preferred metal being complexed is Ni, followed by Cu, Zn, or Pb.

EDTA complexes can undergo metal exchange reactions. Due to this property, non-degradable complex species can be transformed into degradable compounds. However, because of the kinetical stability of the complexes (half-lifes up to 20 days are reported), this process is not likely to occur in municipal treatment plants as the retention time is too short.

EDTA is able to solubilise heavy metal ions previously bound onto sediment solids. It is not possible to give a single value for an EDTA concentration at which no effects on metal remobilisation occurs. Because of the complexity of the EDTA-metal interactions (dependent on metal concentrations, pH, nature of the sediment, concentration of organics etc.), it is not possible to come to a general rule for effects which is applicable to each river system. For individual surface waters, model calculations can be performed to receive a rough estimation. In concentrations below the aquatic PNEC (2.2 mg/l), natural surface waters contain a stoichiometric excess of heavy metal ions, leading to a complete complexation of EDTA in the water phase, thus this effect will not lead to an increase of the total heavy metal level. Only metal exchange reactions can occur.

3.1.2.4 Regional exposure

For the regional exposure assessment it is assumed that the total EDTA consumption volume is emitted into the environment. Degradation in treatment plants or waste deposition/ incineration was not considered as the respective amounts are not known. The resulting regional PECs are 95 µg/l for surface waters and 22 µg/kg for agricultural soil.

3.1.3 Atmosphere

At some production sites, EDTA dust is released into the atmosphere. For the strongest point source, a PECair of 6.7 $\mu g/m^3$ is calculated.

3.1.4 Terrestrial compartment

3.1.4.1 Production

Dust emission during production will reach the soil in the vicinity of the production sites by wet and/or dry deposition. For the strongest emission source, a $PEClocal_{soil}$ of 0.31 mg/kg dw and a $PEC_{porewater}$ of 4.6 µg/l is calculated.

3.1.4.2 Use

Cu-, Fe-, Mn-, Mg-, Mo- and Zn-EDTA complexes are mixed into fertilisers if there is a lack of trace elements in agricultural soil. As a worst-case approach, the exposure from the use as leaf fertiliser was calculated, leading to a $PEClocal_{soil}$ of 0.51 mg/kg dw and a $PEC_{porewater}$ of 7.6 µg/l.

3.1.5 Secondary poisoning

As there is no bioaccumulation, a biomagnification via the food chain is not expected.

3.2 EFFECTS ASSESSMENT

3.2.1 Aquatic compartment (incl. sediment)

The toxicological profile of EDTA is based on disturbances of metal metabolism. For the interpretation of toxicity tests, the complex formation properties of EDTA have to be taken into account. Beside Ca and Mg, test media contain a certain amount of heavy metal ions being necessary as trace nutrients. The complex formation constants of heavy metal complexes are by several orders of magnitude higher than of Ca/Mg-complexes, thus after addition of the test substance EDTA (as acid or Na-salt) the concentration of uncomplexed trace metals decreases drastically. The degree of Ca/Mg complexation is dependent on the amount of added EDTA. Uncomplexed EDTA is only available when it is present in over-stoichiometric concentrations.

Short-term tests on fish reveal that EDTA and Na-EDTA are more toxic in an uncomplexed form. This can only occur if they are available in over-stoichiometric amounts to the chelants. Under these conditions the complexing agents can cause nutrient deficiency by reducing the essential concentration of different ions. The higher the water hardness the higher was the concentration of EDTA necessary to cause a toxic effect expressed as mortality. In the test

result obtained with Na_2EDTA and a water hardness of 103 mg/l CaCO3 (96-hour LC50 = 374 mg/l) pH effects of the acid are completely suspended. However, uncomplexed EDTA was applied in a stoichiometric excess which is in contrast to environmental conditions. Using $CaNa_2EDTA$ as test substance, a LC50 of 1,827 mg/l was obtained being in a concentration range where unspecific effects are expected. All tests on acute fish toxicity are of limited relevance for the PNEC derivation.

In an early-life stage test on the zebrafish *Danio rerio*, the NOEC was determined to > 26.8 mg/l H_4EDTA based on analytically determined concentrations. $CaNa_2EDTA$ was used as test substance. This test is considered to be the most relevant fish test for the PNEC derivation.

For daphnids no investigation on the influence of water hardness or possible reduced nutrient conditions are available. The available acute tests are carried out by Bringmann and Kühn in hard water (160 mg/l CaO). It is known that calcium deficiency inhibited the development of fresh water crawfish. 24-hour EC50 values of 480 to 790 mg/l for Daphnia magna were found. In a long-term test a 21-day NOEC of 22 mg/l for reproduction could be obtained. In the latter test a surplus of Ca was present, thus mainly Ca-EDTA was formed in the medium being the active test substance.

The apparent effects of complexing agents to algal growth are related to essential trace metal bioavailability. It was demonstrated that not the absolute EDTA concentration, but rather the ratio of the EDTA concentration to the metal cations is crucial to algae growth. With sufficient trace metal amounts, H_4EDTA concentrations up to 310 mg/l caused no effects. Similar results are obtained when Fe(III)EDTA is used as test substance, due to its slow metal exchange kinetics overchelation of the nutrient metal ions is avoided. Therefore direct effects caused by the intrinsic toxicity of EDTA are not expected in surface waters, where in nearly every case a stoichiometric surplus of metal ions is present.

In addition to the discussed adverse effects, like growth inhibition, mortality and immobilisation of EDTA the growth stimulating effects like eutrophication occurs. For two different river waters a significant increase in phytoplankton production was observed after addition of 30 to 300 µg/l EDTA. The higher availability of trace elements through the complexing agent EDTA depends on the preloading of the water and can significantly stimulate the processes of eutrophication. If trace elements like Fe, Co, Mn, and Zn are sufficiently available in a soluble form, the algae growth will be increased after addition of EDTA only insignificantly. This aspect of effects can not be assessed quantitatively with the methods available.

3.2.1.1 Determination of $PNEC_{aqua}$

The effects assessment of EDTA is based on long-term tests, which are available for fish, daphnids and algae. The most sensitive endpoint could be found for *Daphnia magna* with a NOEC of 22 mg/l H_4EDTA. According to the TGD an assessment factor of 10 has to be used, leading to a $PNEC_{aqua}$ of 2.2 mg/l.

In the frame of the marine risk assessment there is no scenario for intermittent release available. As there are no valid test results with marine organisms available, it is proposed according to the TGD, Chapter "marine risk assessment" to use an AF of 1,000 instead of 100 for PNECintermittent-marine. Using this factor a $PNECintermittent_{marine}$ of 0.64 mg/l can be calculated.

3.2.1.2 Microorganisms

A respiration test with activated sludge collected from a domestic sewage treatment plant and CaEDTA as the active test substance showed no inhibition of the respiration rate in concentrations up to 500 mg/l. With an assessment factor of 10, a $PNEC_{microorganism}$ of >50 mg/l is obtained.

3.2.1.3 Influence on the toxicity of heavy metals

In surface waters, EDTA causes an increase of heavy metals in the water phase. The influence of EDTA on the toxicity of heavy metals was demonstrated in a test on Daphnia. The toxicity of most metals was decreased by a factor of 17 to 1,700, except with mercury, for which a different toxicity mechanism is assumed.

3.2.1.4 Ecotoxicity of EDTA metabolites

The toxicity of ketopiperazine diacetate (KPDA) was tested on the zebrafish *Brachidanio rerio,* on *Daphnia magna,* and on the alga *Pseudokirchnerella subcapitata.* For all 3 taxonomic groups tested, the EC50 values for ketopiperazine diacetate (KPDA) are above 100 mg/l. With an assessment factor of 1,000, for KPDA a PNEC of > 100 µg/l is calculated.

3.2.2 Atmosphere

Because there are no fumigation tests available, an effects assessment for this compartment can not be performed.

3.2.3 Terrestrial compartment

There are only test results available which investigate the decrease of heavy metal toxicity caused by EDTA. It is not possible to derive a PNEC with this data. Therefore, the assessment can be based on the pore water concentration only.

3.2.4 Secondary poisoning

As there is no bioaccumulation, a biomagnification via the food chain is not expected.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

The risk assessment for aquatic organisms resulted in a $PNEC_{aqua}$ of 2.2 mg/l. The $PNEC_{microorganism}$ was determined to >50 mg/l. For intermittent releases occurring at oil platforms, the $PNEC_{intermittentmarine}$ was determined to 0.64 mg/l.

Site-specific scenarios

Table 3.1 PEC/PNEC ratios for the aquatic compartment

Scenario	$PEClocal_{aqua}$ [mg/l]	PEC_{aqua} / $PNEC_{aqua}$	Ceffl [mg/l]/ Ceffl	Ceffl. / $PNEC_{microorganism}$
Producer A	only import			
Producer B	0.18	0.08	9.7	<0.19
Producer C	0.095	0.04	0.040	<0.0008
Producer D	max. 1.0	<0.45	no WWTP	-
Producer E *	0.36	0.16	1,500	<30
Producer F	0.10	0.045	no WWTP	-
Producer G	0.22	0.1	no WWTP	-
Producer H	0.098	0.04	0.40	< 0.008
Household sewage	0.195	0.09	1.0	< 0.02
Industrial detergents Scenario 1	0.64	0.29	5.4	< 0.11
Industrial detergents Scenario 2	2.6	1.2	25	< 0.5
Industrial detergents Scenario 3	0.35	0.16	2.5	< 0.05
Photochemicals	0.57	0.26	4.7	< 0.09
Textile industry	2.0	0.9	19	< 0.38
Pulp and paper Scenario 1	0.5	0.23	4.0	< 0.08
Pulp and paper Scenario 2	2.6	1.2	40	< 0.8
Metal plating	12	5.5	116	< 2.3
Polymer and rubber production	1.7	0.77	16	< 0.32
Disposal	2.4	1.1	23	< 0.46

* At site E production was meanwhile stopped

A **conclusion (iii)** was reached for the following scenarios:

- for the use of EDTA in industrial detergents by large sites within dairy and beverage industry, where no effective wastewater treatment is applied,
- for paper mills where no effective wastewater treatment is applied,
- for metal plating (circuit board production),
- for releases at waste disposal sites.

Influence on the distribution of heavy metals

In high concentrations (which can occur when strong point sources are emitting into a small river) EDTA prevents the adsorption of heavy metals onto sediments and can remobilise metals from highly loaded sediments. Both effects lead to increased heavy metal concentrations in the water phase. On the other hand, the aquatic effects assessment resulted that the EDTA complexes of heavy metals are less toxic than the uncomplexed metals. Overall, a risk for the aquatic environment due to the influence of EDTA on the mobility of heavy metals is not expected. **Conclusion (ii)**.

EDTA metabolites

The sum of ketopiperazinediacetate (KPDA) and ethylendiaminetriacetic acid (ED3A) was detected in German rivers and drinking water in concentrations of 0.5 to 16 µg/l. From tests on acute toxicity, a PNEC of > 100 µg/l for KPDA was derived. Assuming that, because of the similar molecular structure, ED3A has a similar toxicity as EDTA (PNEC = 2.2 mg/l), the environmental concentrations are far below both PNECs, thus a risk is not expected.

Further metabolites like ethylenediaminediacetic acid (N,N-EDDA and N,N'EDDA) and ethylenediaminemonoacetic acid (EDMA) are either photolysed or more rapidly biolocical degraded than the mother substance EDTA. Therefore, their environmental concentrations are assumed to be lower than the calculated PECs for EDTA. Because of the similar molecular structure, their ecotoxicity is assumed to be similar (or at least not much higher); therefore a risk from these substances is not expected. **Conclusion (ii)**.

3.3.2 Atmosphere

EDTA is emitted into the atmosphere in dust form during production. The $PEClocal_{air}$ for the strongest emission source was estimated to 6.7 µg/m³. No appropriate effect tests are available, so a risk characterisation ratio for this compartment can not be calculated. However, because of the relative low toxicity of EDTA, a risk to the environment is not expected. **Conclusion (ii)**.

3.3.3 Terrestrial compartment

Because there are no effect tests on terrestrial organisms available, the risk characterisation is based on the calculated porewater concentrations.

During production, EDTA is emitted into the atmosphere at several sites. Deposition into soil resulted in a porewater concentration of maximum 4.6 µg/l. With a $PNEC_{aqua}$ of 2.2 mg/l, the PEC/PNEC ratio is 0.002.

The use of EDTA as leaf fertiliser was regarded as a worst-case scenario for the exposure estimation, resulting in a $PEC_{porewater}$ of 7.6 µg/l. With a $PNEC_{aqua}$ of 2.2 mg/l, the PEC/PNEC ratio is 0.003.

For both scenarios, a risk to terrestrial organisms is not expected. **Conclusion (ii)**.

3.3.4 Secondary poisoning

As there is no bioaccumulation, a biomagnification via the food chain is not expected. **Conclusion (ii)**.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 Occupational exposure

The exposure assessment for workers is made for both substances, H_4EDTA and Na_4EDTA, summarised as EDTA. The uses of EDTA are determined by its high capacity for complexing metal ions. Diluted substances with EDTA concentrations below 5 % are mainly used, e.g. as detergents (household, industrial), in photochemicals, in agriculture products and in cosmetics.

Detailed information on the production volumes is given in Section 2.

Based on the available information the following relevant occupational exposure scenarios are to be expected:

- production and further processing as a chemical intermediate (Scenario 1),
- formulation of preparations (Scenario 2),
- uses of formulations including formulation of preparations on-site (Scenario 3).

Occupational exposure limits (OEL) have not been established.

The exposure assessment is based on measured data and literature data, expert judgement and estimations according to the EASE model (Estimation and Assessment of Substance Exposure). The exposure levels should be regarded as reasonable worst-case estimates representing the highly exposed workers.

The results for the different scenarios are summarised in **Table 4.1**. More detailed information on inhalation and dermal exposure is given below.

Inhalation Exposure

For the large-scale chemical industry, it is assumed that the production and further processing of EDTA is mainly performed in closed systems with high levels of protection. Most of the substance is produced in a liquid form. Due to the physico-chemical properties of the substance (low-vapour pressure), inhalation exposure to vapour during the handling of solutions is assessed as negligible.

Higher exposures are expected if EDTA is produced or used as powders. During the production exposure of dusts occurs if the closed systems are breached for certain activities e.g. filling (Scenario 1). If the formulation of products is performed using the powdery substance, possibilities of inhalation exposure occur during weighing and filling (Scenario 2).

Inhalation exposure has to be considered if droplet aerosols are formed during the application of aqueous preparations, e.g. high pressure cleaning, metal-cutting with cooling lubricants, pre-treatment of metal, electroplating industry, use of herbicides and insecticides. Among the different fields of applications high-pressure cleaning is considered to be the most important exposure scenario (Scenario 3). Inhalation exposure is negligible if aerosols are not formed.

Dermal Exposure

With regard to dermal exposure, measured results are not available. For most occupational exposure scenarios, the regular use of suitable PPE (Personal Protective Equipment) at the workplaces is not probable. Therefore, actual dermal exposure is generally assessed based on the EASE model without considering that PPE might be worn by a part of the exposed collective. In general, dermal exposure is assessed as exposure to part of hands and forearms.

Here on account of the low dermal absorption of the substance (0.001 %), dermal exposure is regarded to be of minor relevance for occupational risks. As a theoretical worst-case estimate might serve an EASE estimation for wide dispersive use and intermittent contacts leading to a dermal exposure level of 5 mg/cm²/day. Considering an exposed area of 840 cm², dermal exposure is assessed to 4,200 mg/person/day. The calculated internal body burden (4,200 ·0.001% / 70) of 0.6 µg/kg/day is far below the internal NAEL (no adverse effect level) of 21.8 mg/kg/day for H_4EDTA (28.2 mg/kg/day for Na_4EDTA). Taking into account that the resulting MOS (margins of safety) of > 36,000 is clearly beyond concern, and therefore dermal exposure is not assessed quantitatively.

Summary of exposure data

Table 4.1 Summary of exposure data

Exposure scenario	Duration and frequency of activities relevant for exposure	Inhalation exposure Shift average [mg/m³]	Dermal exposure Shift average [mg/p/day]
Production and further processing in the chemical industry			
1) Production and further processing of powdery EDTA	shift length, daily	2 – 5 [1), 2)]	[5)]
Preparation and use of formulations			
2) Preparation of formulations, handling of the powdery substance	1 hour (assumed), daily	a) 0.3 [3)](with LEV) b) 0.6 [3)](without LEV)	[5)]
3) High-pressure cleaning (diluted solutions, < 2% EDTA)	4 hours (assumed), daily	0.3 [4)] determined by analogy	[5)]

LEV – local exhaust ventilation

1) Exposure assessment based on model estimates (EASE model), estimate supported by measured results
2) Most of the substance is produced as a liquid formulation. In this case inhalation exposure is assessed as negligible.
3) Due to the low quantities of the substance used, the lower exposure levels of the assessed ranges are taken for risk assessment.
4) Exposure assessment exemplary for all uses of formulations (liquid, powdery) where the formation of aerosols is possible. Exposure is negligible, if aerosols are not formed.
5) On account of the low dermal absorption of the substance (0.001 %), dermal exposure is regarded to be of minor relevance for occupational risk assessment.

4.1.1.2 Consumer exposure

The most important exposure of consumers to EDTA results from use of household detergents and cosmetics. The use in textiles can not be estimated because of lacking data. EDTA is used as a component of cosmetics (skin creams and lotions, after care products for hair in concentrations of <0.2%, hair bleaches in concentrations of 1%, washing gels in concentrations of <0.01%), and of cleansing agents, dish washing agents in concentrations of <0.5%, and cleansing agents for orthodontic devices in concentrations of <10%.

Furthermore, it might be assumed that the consumer is exposed to EDTA by the oral route due to migration of the substance from plastics coming into contact with foods. An estimation of this exposure is not possible due to the lack of data about the amounts used for this purpose and the migration rate.

Dermal exposure to cosmetics

Assuming the use of 16 g of a lotion containing 0.2% of EDTA per day, the total exposure can be estimated to about 0.5 mg/kg bw/day.

For hair bleaching the use of an amount of 5-6 ml (≈ 5-6 g) of a hair bleaching agent containing 1% of EDTA is assumed. Taking into account the retention factor of 10, than 6 mg of EDTA would contact skin, which corresponds to 0.1 mg/kg bw.

Dermal exposure to household cleansers or dish washing agents

To estimate the exposure of consumers to household cleansers the value of weight fraction was set to 10% as a worst-case estimate to cover all of the subcategories of household cleansers. This is the highest concentration reported among 427 products in the BgVV database. The most common concentration of EDTA in household cleaners, however, is below 1%. The worst-case estimate of the daily dermal exposure was calculated to be 0.12 mg/kg bw/day.

The calculation of the total dermal exposure of consumers results in a value of about 0.72 mg/kg bw/day.

Oral exposure

Oral exposure may result from the use of cleansers of tooth brackets that contain maximum concentrations of 5% of EDTA. As a worst-case, the residual amount of EDTA on a bracket would be 3 mg. Because tooth brackets are normally used in childhood, the calculation was related to the bodyweight of a 10 year old child, (30 kg; 5th percentile), and the resulting exposure amounts to be 0.1 mg/kg bw/day.

4.1.1.3 Humans exposed via the environment

The only significant indirect exposure for human occurs via drinking water. A significant intake via fish, plants or meat is not expected because EDTA does not accumulate in biota. Model calculations have been performed for the local scenario for the different producers resulting in a total daily dose of EDTA in drinking water in the range from 0.003 to 0.38 mg/kg bw/day. For the regional scenario a total daily dose of 0.0039 mg/kg bw/day was calculated.

4.1.2 Effects assessment

Justification for cross-reading from different EDTA compounds

In general, edetic acid (H_4EDTA) and tetrasodium EDTA show similar properties and exposure pattern. However, with respect to acute toxic and local effects both substances behave differently. Thus, the hazard effects of the two substances are evaluated separately for the endpoints acute toxicity, irritation, corrosivity and sensitisation based on the test substances used in the respective toxicity assays. For systemic effects studies with administration of H_4EDTA or of its salts such as Na_2H_2EDTA, Na_3HEDTA and Na_4EDTA

were considered as relevant information because these compounds are dissociated under physiological conditions (pH 7 - 9) into the sodium cations and the respective anionic species of edetic acid ($HEDTA^{3-}$) depending on the pH-dependent dissociation equilibria of edetic acid. Taken together, any conclusions on H_4EDTA or Na_4EDTA will be derived from consideration of the overall available data base.

Data from studies with the soluble, but strongly associated complex calcium disodium edetate ($CaNa_2EDTA$) were not considered in the report except the sections on toxicokinetics and reproductive toxicity. Taking into account the stability constant of the calcium EDTA complex (about 10^{10} M^{-1}) the concentrations of free anionic EDTA species in $CaNa_2EDTA$ solutions can be estimated to amount < 0.01% according to the mass action law. Thus, almost all proportion of the $CaNa_2EDTA$ complex is still present as $CaEDTA^{2-}$ species, whereas only a very minor proportion of the $CaNa_2EDTA$ complex exists as free anionic EDTA species in solution which is considered to be too low for detecting generally toxic (systemic) effects of EDTA or sodium salts of EDTA. The $CaNa_2EDTA$ will chelate any other metal that has a higher binding affinity than Ca^{2+} (e.g. lead, iron, zinc, and copper). E.g., zinc chelates with $CaNa_2EDTA$ to form a complex that shows a 10^4 times higher binding affinity than that of the calcium complex. Therefore, application of $CaNa_2EDTA$ will result in complexation of zinc ions thus interfering with the zinc homeostasis and leading finally to developmental toxicity.

Toxicokinetics, metabolism and distribution

There are no oral toxicokinetic studies or skin absorption studies with EDTA itself or its tetrasodium salt available. According to the dissociation equilibrium of edetic acid administration of different sodium salts will result in dependence on the intestinal pH-value to the formation of various anionic species of EDTA. It can be assumed that the oral and dermal absorption of sodium salts of EDTA and of the free acid is comparable to the measured low absorption of $CaNa_2EDTA$. It is poorly absorbed from the gastrointestinal tract (a maximum of 5% was detected in the urine). Only 0.001% of $CaNa_2EDTA$ is absorbed after dermal application. In whatever salt EDTA is administered it is likely to chelate metal ions *in vivo*.

Acute toxicity

In rats the acute oral toxicity is low. In two tests LD50 values of > 2,000 mg/kg were reported. There is no need for classification and labelling for acute oral toxicity.

In a test system similar to the inhalation hazard test there was no mortality after an 8-hour exposure of an unknown test concentration of the substance that was heated to either 20 or 80°C. These data are considered to be sufficient for the risk assessment of acute inhalation toxicity. There is no need for classification and labelling for acute inhalation toxicity.

No data are available on acute dermal toxicity. Taking in account the poor dermal absorption it can be assumed that the result of an acute dermal toxicity test would not reveal toxic properties warranting a classification and labelling for acute dermal toxicity.

Irritation

A 50% aqueous preparation of edetic acid (no data on purity) resulted in a mild irritation of the skin after a 20-hour exposure time. It can be concluded that these findings do not warrant a classification and labelling for skin irritation. Instillation of 50 mg of solid edetic acid (no data on purity) to the rabbit eye resulted in strong but reversible irritant effects that warrant a classification as „Xi, Irritant" and labelling as „R 36, Irritating to eyes".

Sensitisation

In a Magnusson Kligman Test with Na_2EDTA according to OECD Guideline 406, 30% of the guinea pigs showed a positive response after a first challenge and 10% after a second challenge. There are only two reports on single cases in humans demonstrating positive skin results. Based on the fact that EDTA is being used in industry and consumer products for many decades in high quantities the substance is considered as non-sensitising to humans. A labelling with R 43 „May cause sensitisation by skin contact" is not warranted. No adverse acute or chronic respiratory health effects from exposure to EDTA or Na_4EDTA have been observed in workers. Taking into account that the result of the study of Beasley et al. (1987) could not be reproduced, a labelling as R 42 „May cause sensitisation by inhalation" is not warranted.

Repeated dose toxicity

From repeated dose toxicity experiments (90-day feed male Holtzmann rats, 2-year bioassay both sexes Fischer 344 rats and B6C3F1 mice) a NOAEL of 500 mg/kg/day for Na_2EDTA and Na_3EDTA could be derived (corresponding to 435 mg/kg bw/day EDTA). Although the 90-day study was performed only in male animals and does not provide a full range of today's clinical biochemistry the data provided information on histopathology mainly from the long term study and parameters such as body weight and some hematological parameters do justify this non toxic effect level. The adverse effects seen with higher-dose levels were increase in mortality, reduced body weight, reduced food consumption, diarrhea, emaciation, and sometimes parakeratosis in oesophagus and forestomac as well as decreased hemoglobin and hematocrit levels.

Mutagenicity

Bacterial mutation tests are negative, but mutations and DNA damage were found in mouse lymphoma cells after exposure to very high concentrations. For somatic cells in mice (bone marrow cells) negative results with respect to the endpoints micronuclei, aneuploidy and sister chromatid exchanges were described. In germ line cells negative results were obtained for induction of structural chromosomal aberrations in spermatogonia, for induction of aneuploidy in primary and secondary spermatocytes, and also for induction of dominant lethals. A positive result was obtained in a micronucleus test with spermatids, indicating that aneugenic effects may be induced in specific phases of spermatogenesis (late spermacytogenesis). The effect was bound to the use of an extremely high dose in the LD50 range. Altogether, EDTA and its sodium salts have a low mutagenic potential at extremely high doses. On the basis of the various negative findings and the assumption of a threshold mode-of action for aneugens, it can be concluded that EDTA and its sodium salts are not mutagenic for humans.

Carcinogenicity

A bioassay of Na_3EDTA for possible carcinogenicity was conducted by administrating of test material in the diet to Fischer 344 rats and B6C3F1 mice. The studies did not report specific data on kidney toxicity in either species. Although a variety of tumours occurred among test and control animals of both species, no tumours were related to treatment. Thus, there is no concern on a carcinogenic potential of EDTA.

Toxicity for reproduction

Concerning reproductive toxicity, valid data from human experience are not available. Data from a multigeneration study in rats with $CaNa_2EDTA$ did not give evidence for adverse effects on reproductive performance and outcome for doses of up to 250 mg/kg bw/day. From a less valid study with Na_2EDTA conducted in rats complete reproductive failure was reported at dietary dose levels of 3,000 mg/kg bw/day.

Developmental toxicity of EDTA, sodium salts and calcium and zinc chelates was investigated in studies in rats, mainly in single dosage studies. After repeated treatment of dams during various periods of gestation and with the use of different routes of substance application (diet, gavage, s.c., i.m.) impaired embryo/fetal development and the induction of a pattern of gross malformations were observed during these investigations with the exception of one study (Schardein et al., 1981)[2]. Gross malformations comprised cleft palate, severe brain deformities, eye defects, micro- or agnathia, syndactyly, clubbed legs and tail anomalies. These effects were almost exclusively exhibited in studies using maternally toxic dosage levels.

It has been repeatedly reported that the pattern of malformations observed after exposure of pregnant female rats to EDTA, edetic acid salts or calcium EDTA is similar to that observed when dams were held on zinc depleted diets during either short intervals or for the whole period of gestation. Since it has been demonstrated that zinc deficient diets per se lead to developmental and teratogenic effects in offspring the depletion of zinc in the diet and/or the depletion of endogenous zinc tissue concentrations caused by EDTA treatment appear to be of specific significance for embryo/fetal impairment and the induction of malformations. With sufficient zinc supplementation fetotoxic and teratogenic effects could be prevented, respectively minimised.

The teratogenic effect of EDTA has been shown to be attributable to an interference with zinc homeostasis in the dams and fetuses. However, in all but one study with the oral route of administration, the doses leading to teratogenic effects are always paralleled by diarrhea, which in turn will additionally increase zinc deficiency. Therefore, it can be discussed whether the teratogenic effect is primarily attributed to unspecific weight reduction in dams or whether this effect is due to specific interference with zinc homeostasis. Fetotoxicity may be as well related to reduced body weight of the dams. The second point to be discussed is the mechanism of action of zinc depletion and hence teratogenicity. Three mechanisms of zinc depletion can contribute to the teratogenic effects: i) reduction of available zinc by complexation in the upper intestine, ii) enhanced urinary excretion, and iii) enhanced zinc excretion into the gut lumen by diarrhea.

The fetotoxic and teratogenic effects are occurring at exposure levels of approximately 1,000 mg/kg bw/day and above. We do not recommend classifying EDTA/Na_4EDTA as being a reproductive toxicant due to the following reasons: i) the malformations have been demonstrated at relatively high oral dose levels and ii) a steep dose response relationship can be assumed. No oral NOAEL for either developmental toxicity or maternal toxicity could be established.

[2] Please refer to the comprehensive Final RAR for the full reference.

4.1.3 Risk characterisation

4.1.3.1 Workers

4.1.3.1.1 Introductory remarks

H_4EDTA and Na_4EDTA are solid substances with water solubilities of 0.4 g/l and 500 g/l at 20 °C. The vapour pressure is assumed to be very low and evaporation is considered to be not relevant. A widespread use of H_4EDTA and Na_4EDTA is described in section 4.1.1.1 and the central scenarios are listed in **Table 4.1**. Since H_4EDTA and Na_4EDTA are assumed to show similar toxicity in several endpoints an integrated assessment is performed. As far as differences in toxicity have to be regarded they are assessed separately. Toxicological key effects are eye irritation and developmental toxicity at very high doses.

Acute toxicity, repeated dose toxicity and developmental toxicity are assessed on the basis of MOS values and oral studies represent the central database for the quantitative assessment of these endpoints. Performing a risk assessment of dermal contact and inhalation exposure a route to route extrapolation has to be performed and systemic availability via all routes has to be considered. The maximum systemic availability after oral application is 5%, while for the dermal route a maximum value of 0.001% is given. For inhalation a worst-case assumption of 100% systemic availability is applied. Because of the negligible skin absorption systemic effects after dermal contact are not considered to be relevant.

As starting points for MOS calculation the oral toxicity data are converted into values of internal body burden, taking the 5% systemic availability after ingestion into account. The MOS values are calculated as quotient of the converted NAEL/LAEL values and the internal body burden from inhalation exposure at the workplace. The minimal acceptable MOS, as decision mark between conclusion (ii) and (iii), results from the multiplicative combination of different factors, which consider e.g. interspecies differences, intraspecies variability and the nature of effect (see comprehensive risk assessment report). Minimal MOS values may be different for each toxicological endpoint pending on the overall database and the effect under assessment. In a parallel procedure an acceptable exposure concentration is identified indicating concern if occupational exposure concentrations exceed this value.

4.1.3.1.2 Occupational risk assessment

Acute toxicity

Inhalation

There are no valid data on acute inhalation toxicity (H_4EDTA and Na_4EDTA). Thus the assessment is based on oral data, starting with an NOAEL of 2,000 mg/kg for H_4EDTA and 565 mg/kg for Na_4EDTA. As described above an internal NAEL of 100 mg/kg is calculated for H_4EDTA and 28 mg/kg for Na_4EDTA. A minimal acceptable MOS of 20 (4 · 5) is used which results in an acceptable internal body burden of 5 mg/kg/day for H_4EDTA (1.4 mg/kg/day for Na_4EDTA). The minimal MOS of 20 is composed of an interspecies factor of 4 and an uncertainty factor of 5, which covers intraspecies variability, nature of effect and dose response relationship. The corresponding exposure concentration assuming a 100% absorption via inhalation is 35 mg/m^3 for H_4EDTA (9.9 mg/m^3 for Na_4EDTA). All MOS values are higher than 20 and concern is not derived. **Conclusion (ii)**.

Dermal

Acute systemic effects after dermal exposure are not considered to be relevant due to the very low skin absorption. **Conclusion (ii)**.

Irritation/Corrosivity

Weak effects have been observed in skin irritation studies of H_4EDTA and Na_4EDTA. This was not sufficient for classification, concern is not derived.

H_4EDTA is considered to be irritating to the eyes. Na_4EDTA may result in serious damage to the eye. Conclusion (ii) is proposed on the grounds that control measures exist which can minimise exposure and risk of irritation, thereby reducing concern. However, these controls must be implemented and complied with to reduce the risk of damage to eyes. **Conclusion (ii)**.

Sensitisation

Dermal

A very weak response was observed in a skin sensitisation test in guinea pigs and only two reports on skin sensitisation in humans are available. Based on the fact that the substance is being used in industry and consumer products for many decades in high quantities the incidence of positive responses is too low to derive concern as to skin sensitisation. **Conclusion (ii)**.

Inhalation

No acute or chronic respiratory health effects have been observed in workers from exposure to EDTA. There is no valid indication for EDTA as a respiratory sensitiser. **Conclusion (ii)**

Repeated dose toxicity

Inhalation (local effects)

Valid inhalation studies with single or repeated exposure in animals are not available (H_4EDTA and Na_4EDTA). The eye irritation points to an irritative potential, but there are no reports of effects in humans, that might confirm a potential risk. No concern is derived. **Conclusion (ii)**.

Inhalation (systemic effects)

For the assessment of systemic effects after repeated inhalation the repeated oral studies are taken into account. Subchronic and chronic toxicity studies in rodents resulted in a NOAEL of about 500 mg/kg/day. At higher doses adverse effects like diarrhoea and emaciation were observed. The NOAEL of 435 mg/kg/day for H_4EDTA (565 mg/kg/day for Na_4EDTA) is used for risk assessment.

An internal NAEL of 21.8 mg/kg/day is calculated for H_4EDTA and 28.2 mg/kg/day for Na_4EDTA. A minimal MOS of 20 (4 · 5) is used which results in an acceptable internal body burden of 1.1 mg/kg/day for H_4EDTA (1.4 mg/kg/day for Na_4EDTA). The minimal MOS of 20 is composed of an interspecies factor of 4 and an uncertainty factor of 5, which covers intraspecies variability, nature of effect and dose response relationship. The corresponding acceptable exposure concentration assuming a 100% absorption via inhalation is 7.4 mg/m^3

for H_4EDTA (9.9 mg/m^3 for Na_4EDTA). All MOS values are higher than 20 and concern is not derived. **Conclusion (ii)**.

Dermal (local effects)

See under "Irritation/Corrosivity/Dermal", no further information available. **Conclusion (ii)**

Dermal (systemic effects)

Chronic systemic effects after dermal exposure are not considered to be relevant due to the very low skin absorption. **Conclusion (ii)**.

Mutagenicity and Carcinogenicity

H_4EDTA and Na_4EDTA are not considered to be mutagens in humans. Based on the results of oral long-time studies in rats and mice, H_4EDTA and Na_4EDTA are not considered to be carcinogenic. **Conclusion (ii)**.

Reproductive toxicity (fertility impairment)

No evidence for adverse effects on fertility were observed in a rat multigenerational study conducted with $CaNa_2EDTA$ up to the highest tested dose of 250 mg/kg/day. Effects on the reproductive organs are also not reported in subchronic and chronic studies in rodents. Based on these data H_4EDTA and Na_4EDTA are not considered to impair fertility. **Conclusion (ii)**.

Reproductive toxicity (developmental toxicity)

Inhalation

It is assumed that developmental toxicity of EDTA is based on its metal chelating capacity and especially on endogenous zinc depletion. In rats high oral doses of EDTA (as Na_2H_2EDTA) led to fetotoxicity and teratogenicity accompanied by maternal toxicity. The maternal and fetal LOAEL was approximately 1,000 mg/kg/day (application with the diet). An internal LAEL of 50 mg/kg/day is calculated for EDTA. A minimal MOS of 60 is applied which results in an acceptable internal body burden of 0.83 mg/kg/day. The minimal MOS of 60 is composed of an interspecies factor of 4 and an uncertainty factor of 15, which covers intraspecies variability, nature of effect and the dose response relationships of the different studies on developmental toxcity. The corresponding acceptable exposure concentration assuming a 100% absorption via inhalation is 5.8 mg/m^3. Scenario 1 (production and further processing of powdery EDTA, see **Table 4.1**) with the lowest MOS of 70 is considered as a borderline scenario, but having in mind, that worst-case assumptions led to the 100% value of systemic availability after inhalation, concern is not derived. In summary, conclusion (ii) is recommended for all scenarios. **Conclusion (ii)**.

Dermal

Developmental toxicity after dermal exposure is not considered to be relevant due to the very low skin absorption. **Conclusion (ii)**.

4.1.3.1.3 Summary of conclusions for the occupational risk assessment

The occupational risk assessment of dermal and inhalation exposure comes to the conclusion that there is no need for further information and/or testing or for risk reduction measures beyond those which are being applied already (overall conclusion (ii)).

4.1.3.2 Consumers

Exposure

EDTA is used as a component of cosmetics and of cleansing and dish washing agents. Thus, the main route of potential consumer exposure is via dermal contact/absorption through the skin. The calculation of the daily dermal exposure of consumers results in a value of about 0.72 mg/kg bw/day. Taking the experimental data it is assumed that the amount absorbed after dermal exposure will be 0.001% as given by human studies. Thus, the internal exposure from dermal contact may result in a maximum amount of 0.0000072 mg/kg bw/day.

Oral exposure may result from the use of cleansers of tooth brackets if they are not properly cleaned after use. The oral exposure for tooth brackets wearing children amounts to be 0.1 mg/kg bw/day.

Acute Toxicity

Following the exposure assessment, the consumer may be exposed to EDTA via dermal and oral routes. Consumers are not expected to be exposed to EDTA in the range of hazardous doses which can be derived from the oral animal LD50 values (> 2,000 mg/kg bw). Taking into account all assumptions being applied in the exposure estimation scenarios, exposure by inhalation should be considered as of no concern for the consumer. Therefore, the substance is of no concern in relation to acute oral or dermal toxicity. **Conclusion (ii)**.

Irritation / Corrosivity

The substance has weak irritant properties on rabbit skin but has irritant properties to the rabbit eye. The risk for consumers, however, related to ocular exposure is low, given the low levels of EDTA contained in consumer products. According to the dermal exposure scenarios (cosmetics, household cleansers or dish washing agents, reasonable worst case) it can be assumed that irritant concentrations of the substance will not occur. Thus it is concluded that EDTA is of no concern for consumers in relation to possible irritating effects. **Conclusion (ii)**.

Sensitisation

In a Magnusson Kligman Test with Na_2EDTA 30% of the guinea pigs showed a positive response after the first challenge and 10% after a second challenge. The low result of the second challenge does not support an immunologically mediated mechanism. There are only two reports on single cases in humans demonstrating positive skin results. Based on the fact that the substance is being used in industry and consumer products for many decades in high quantities the incidences of positive responses can be considered as very low. Even taking into account the broad consumer exposure via cosmetics and cleansing and dish washing agents, EDTA is considered as non-sensitising to humans. **Conclusion (ii)**.

Repeated dose toxicity

Oral application of Na_2EDTA in the diet to rats for 1 month revealed a NOAEL of 1,125 mg/kg/day (2.25%) in diet. From a 90-day investigation in rats a NOAEL of 500 mg/kg/day equivalent to 1% in diet can be deduced for male rats. This dose corresponds to 435 mg/kg bw/day edetic acid. The adverse effects seen were increase in mortality, reduced body weight, reduced food consumption and diarrhea. Investigations with Na_3EDTA over a period of two years in rats and mice revealed a NOAEL of 500 mg/kg/day hence supporting the NOAEL of 435 mg/kg/day seen in the 90-day study.

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The substance has weak irritant properties on rabbit skin but has irritant properties to the rabbit eye. The risk for consumers, however, related to ocular exposure is low, given the low levels of EDTA contained in consumer products. According to the dermal exposure scenarios (cosmetics, household cleansers or dish washing agents, reasonable worst case) it can be assumed that irritant concentrations of the substance will not occur. Thus it is concluded that EDTA is of no concern for consumers in relation to possible irritating effects. **Conclusion (ii)**.

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In a Magnusson Kligman Test with Na_2EDTA 30% of the guinea pigs showed a positive response after the first challenge and 10% after a second challenge. The low result of the second challenge does not support an immunologically mediated mechanism. There are only two reports on single cases in humans demonstrating positive skin results. Based on the fact that the substance is being used in industry and consumer products for many decades in high quantities the incidences of positive responses can be considered as very low. Even taking into account the broad consumer exposure via cosmetics and cleansing and dish washing agents, EDTA is considered as non-sensitising to humans. **Conclusion (ii)**.

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Dermal exposure

The margin of safety for dermal exposure between the assumed internal exposure level of 0.0000072 mg/kg bw/day and the oral NOAEL (EDTA) of 21.8 mg/kg bw/day is judged to be sufficient, even if special considerations e.g. the nature and severity of the effects are taken into account. The oral NOAEL of 435 mg/kg bw/day has been converted into an internal value of 21.8 mg/kg bw/day considering the maximum oral absorption of 5%. It is concluded therefore that EDTA is of no concern for consumers in relation to the use cosmetics and household detergents. **Conclusion (ii)**.

Oral exposure

The oral exposure of children (via cleansers for tooth brackets) has been calculated to be 0.1 mg/kg bw/day. The margin of safety between the exposure level of 0.1 mg/kg bw/day and the oral NOAEL (EDTA) of 435 mg/kg bw/day is judged to be sufficient, even if special considerations e.g. the nature and severity of the effects are taken into account. It is concluded therefore that EDTA is of no concern for children in relation to use of cleansers for tooth brackets **Conclusion (ii)**.

Mutagenicity

EDTA shows a low mutagenic potential at extremely high doses. On the basis of the various negative findings and the assumption of a threshold mode of action for aneugens, it can be concluded that EDTA is not mutagenic for humans. **Conclusion (ii)**.

Carcinogenicity

There is no evidence on carcinogenic properties of Na_3EDTA from studies in experimental animals. Therefore it can be concluded that EDTA is of no concern for consumers. **Conclusion (ii)**.

Reproductive toxicity (fertility impairment)

Data from a multigeneration study on rats with $CaNa_2EDTA$ did not give evidence for adverse effects on reproductive performance and outcome for doses of up to 250 mg/kg bw/day. Hence the NOAEL is 250 mg/kg bw/day corresponding to 196 mg/kg bw/day edetic acid. This oral NOAEL has been converted into an internal value of 9.8 mg/kg bw/day considering the maximum oral absorption of 5%. The margin of safety for dermal exposure between the assumed internal exposure level of 0.0000072 mg/kg bw/day and the oral NOAEL (EDTA) of 9.8 mg/kg bw/day is judged to be sufficient. Thus, EDTA is of no concern for consumers to affect the reproductive performance. **Conclusion (ii)**.

Reproductive toxicity (developmental toxicity)

The studies with Na_2EDTA form the basis for the estimation of LOAEL of 1,000 mg/kg bw/day. This oral LOAEL been converted into an internal value of 50 mg/kg bw/day considering the maximum oral absorption of 5%. The margin of safety for dermal exposure between the assumed internal exposure level of 0.0000072 mg/kg bw/day and the oral LOAEL (EDTA) of 50 mg/kg bw/day is judged to be sufficient, even if special considerations on the nature and severity of the effects as well as the lack of an established NOAEL are taken into account. Thus, EDTA is considered without concern for consumers with regard to fetotoxic and teratogenic effects. **Conclusion (ii)**.

4.1.3.3 Humans indirectly exposed via the environment

The only significant indirect exposure for human occurs via drinking water. Model calculations have been performed for the local scenario for the different producers which resulted in a total daily dose in the range from 0.003 to 0.38 mg/kg bw/day. For the regional scenario a total daily dose of 0.0039 mg/kg bw/day was calculated. For the purpose of risk characterisation the highest dose of 0.38 mg/kg bw/day will be used. Thus, the margins of safety expressed by the magnitude between the calculated exposures and the NOAEL/LOAEL are considered to be valid for both the local and the regional scenario.

Repeated dose toxicity

From different repeated dose toxicity studies (2-year; 90 day-study's) with mice and rats with sodium salts of EDTA a NOAEL of 500 mg/kg bw/day was derived (respectively 435 mg/kg bw/day EDTA). The margin of safety between the calculated maximum exposure for the indirect exposure source drinking water (0.38 mg/kg bw/day) and the NOAEL (435 mg/kg bw/day) is judged to be sufficient. Thus, regarding repeated dose effects the substance is of no concern in relation to indirect exposure via the environment **Conclusion (ii)**

Fertility (same presentation as for workers and consumers)

Data from a multigenerational study on rats with $CaNa_2EDTA$ did not give evidence for adverse effects on reproductive performance and outcome for doses of up to 250 mg/kg bw/day (respectively 196 mg/kg bw EDTA). The margin of safety between the calculated exposure for the indirect exposure source drinking water (0.38 mg/kg bw/day) and the NOAEL (196 mg/kg bw/day) is judged to be sufficient. Thus, regarding adverse effects on reproductive performance the substance is of no concern in relation to indirect exposure via the environment. **Conclusion (ii)**.

Developmental toxicity (same presentation as for workers and consumers)

Fetotoxic and teratogenic effects occurred in rats at Na_2EDTA exposure levels of approximately 1,000 mg/kg bw/day (LOAEL) and above. The margin of safety between the calculated exposure for the indirect exposure source drinking water (0.38 mg/kg bw/day) and the LOAEL of 1,000 mg/kg bw/day is judged to be sufficient. Thus, regarding fetotoxic and teratogenic effects the substance is of no concern in relation to indirect exposure via the environment. **Conclusion (ii)**.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

Conclusion (ii)

5 RESULTS

5.1 ENVIRONMENT

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached because of the high emissions due to the use of EDTA in industrial detergents. The exposure near sites within dairy and beverage industry with no effective EDTA removal in their treatment plants is expected to lead to a risk for aquatic organisms.

The EDTA exposure for paper mills was estimated on the basis of monitoring data. A high exposure is expected in the receiving water of some sites. Several companies are known to plan long-term aerated biological treatment plants which will reduce the releases.

A high exposure is expected by circuit board producers which have no effective wastewater purification leading to a risk for aquatic organisms.

In the frame of the present risk assessment, it was not possible to gain site-specific information about environmental releases for recovery of EDTA containing wastes. Therefore, the exposure model for the recovery of photochemical based on default values was used for the risk characterisation which leads to a risk for aquatic organisms.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

5.2.1.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.1.2 Consumers

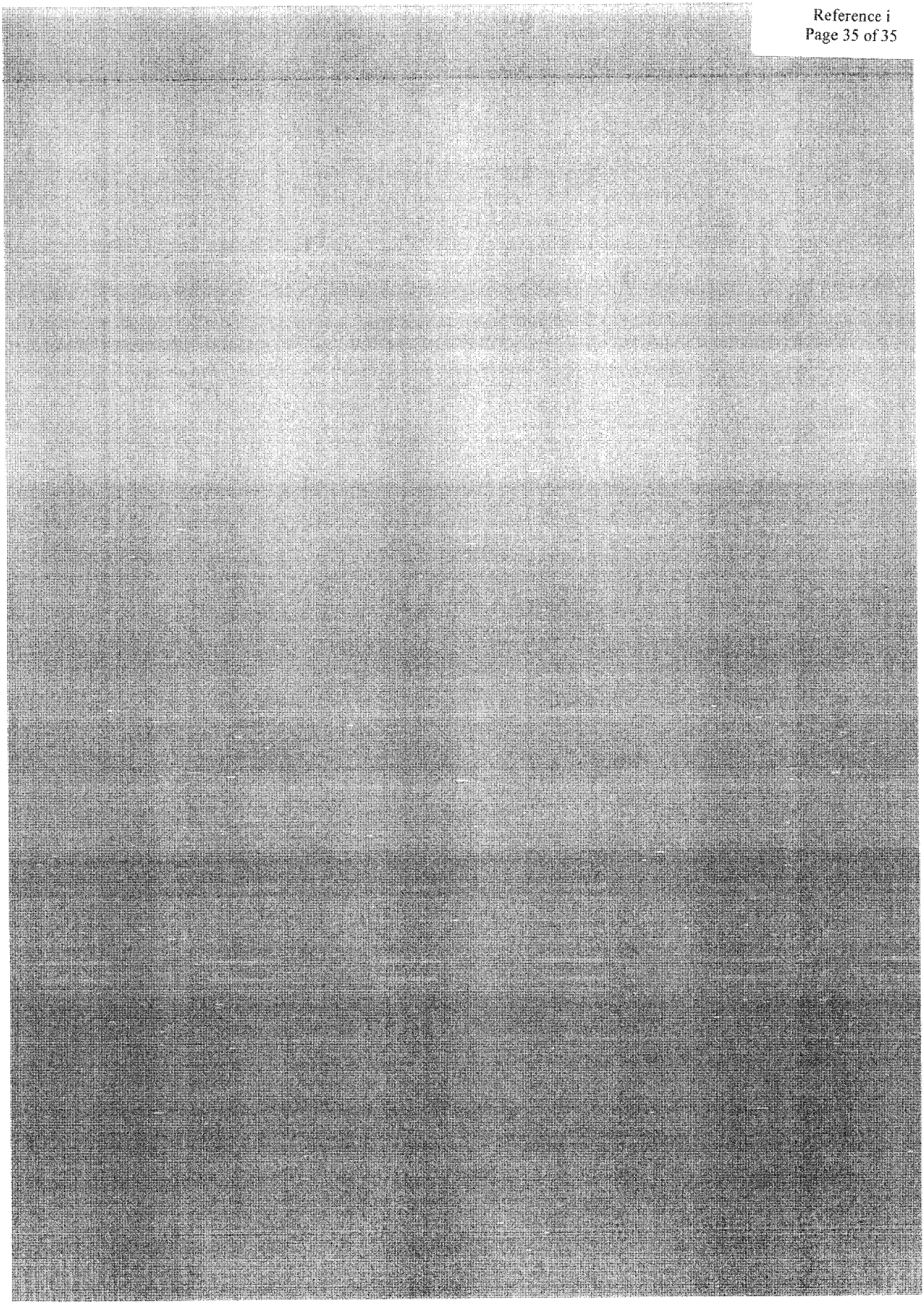
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.



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- Chemical Safety & Handling
- Occupational Exposure Standards
- Manufacturing/Use Information
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First published: 01 January 1994
Metadata| **Permanence level:** Permanent: Stable Content

ETHYLENEDIAMINE TETRAACETIC ACID

CASRN: 60-00-4

For other data, click on the Table of Contents

Human Health Effects:

Human Toxicity Excerpts:

/HUMAN EXPOSURE STUDIES/ ... The free acid or the sodium salt of ... /ethylenediamine tetraacetic acid/ are rather toxic ... the less toxic calcium disodium form ... is more frequently used.
[International Labour Office. Encyclopedia of Occupational Health and Safety. Vols. 1&II. Geneva, Switzerland: International Labour Office, 1983., p. 443]**PEER REVIEWED**

/SIGNS AND SYMPTOMS/ Rapid intravenous administration of Na2EDTA causes hypocalcemic tetany. However, a slow infusion (15 mg/min) administered to a normal individual elicits no symptoms of hypocalcemia because of the ready availability of extracirculatory stores of calcium.
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1868]**PEER REVIEWED**

/CASE REPORTS/ Three cases of ethylenediamine tetraacetic acid (EDTA)-induced leukoagglutination noted on peripheral blood films are reported.
[Deol I et al; Am J Clin Pathol 103 (3): 338-40 (1995)]**PEER REVIEWED**

/OTHER TOXICITY INFORMATION/ ...Total loss of sperm motility was achieved at a concentration of about 5 mg/mL of EDTA. EDTA decreased in vitro percentage of motile sperm as a function of exposure time. It completely impeded sperm penetration into cervical mucus at a concentration much lower than that for 100% inhibition of sperm motility. In the presence of extra calcium, the first-order rate constant for immobilization of sperm was reduced by 38.9-55.6% of control and was inversely proportional to calcium concentration. After EDTA exposure, calcium ion concentration in semen was reduced as a function of EDTA concentration. A decrease of 65% in calcium ion concentration was the threshold at which total loss of sperm motility occurred.
[Lee CH et al; J Pharm Sci 85 (6): 649-54 (1996)]**PEER REVIEWED**

Drug Warnings:

... direct contact with EDTA may cause dermal sensitization (eczema) or allergic conjunctivitis.
[Gesellschaft Deutscher Chemiker (GDCh) - Advisory Committee on Existing Chemicals of Environmental Relevance (BUA); S. Hirzel Verlag, P.O. Box 10 10 61, 70009 Stuttgart, Germany, 1997. xxi, 223p. Bibl.ref.]**PEER REVIEWED**

Probable Routes of Human Exposure:

NIOSH (NOES Survey 1981-1983) has statistically estimated that 509,055 workers (315,637 of these are female) are potentially exposed to ethylenediamine tetraacetic acid in the US(1). Occupational exposure to ethylenediamine tetraacetic acid may occur through inhalation and dermal contact with this compound at workplaces where ethylenediamine tetraacetic acid is produced or used(SRC). Monitoring data indicate that the general population may be exposed to ethylenediamine tetraacetic acid via ingestion of food and drinking water, and dermal contact with this compound and other consumer products containing ethylenediamine tetraacetic acid(SRC).
[(1) NIOSH; National Occupational Exposure Survey (NOES) (1983)]**PEER REVIEWED**

Average Daily Intake:

The average daily intake of ethylenediamine tetraacetic acid for a man who drinks two liters of water per day would be a maximum of 50 ug. The acceptable daily intake for man is 0 to 2.5 mg/kg(1).
[(1) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992)]**PEER REVIEWED**

Emergency Medical Treatment:

Emergency Medical Treatment:

EMT Copyright Disclaimer:

The following Overview, *** GENERAL OR UNKNOWN CHEMICAL ***, is relevant for this HSDB record chemical.

Life Support:

- This overview assumes that basic life support measures have been instituted.

Clinical Effects:

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0.2.1 SUMMARY OF EXPOSURE
 0.2.1.1 ACUTE EXPOSURE
   A)  A SPECIFIC REVIEW on the clinical effects and treatment
       of individuals exposed to this agent HAS NOT YET BEEN
       PREPARED. The following pertains to the GENERAL
       EVALUATION and TREATMENT of individuals exposed to
       potentially toxic chemicals.
   B)  GENERAL EVALUATION -
    1)  Exposed individuals should have a careful, thorough
        medical history and physical examination performed,
        looking for any abnormalities. Exposure to chemicals
        with a strong odor often results in such nonspecific
        symptoms as headache, dizziness, weakness, and nausea.
   C)  IRRITATION -
    1)  Many chemicals cause irritation of the eyes, skin, and
        respiratory tract. In severe cases respiratory tract
        irritation can progress to ARDS/acute lung injury,
        which may be delayed in onset for up to 24 to 72 hours
        in some cases.
    2)  Irritation or burns of the esophagus or
        gastrointestinal tract are also possible if caustic or
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irritant chemicals are ingested.
D) HYPERSENSITIVITY -
1) A number of chemical agents produce an allergic hypersensitivity dermatitis or asthma with bronchospasm and wheezing with chronic exposure.

Laboratory:

A) A number of chemicals produce abnormalities of the hematopoietic system, liver, and kidneys. Monitoring complete blood count, urinalysis, and liver and kidney function tests is suggested for patients with significant exposure.
B) If respiratory tract irritation or respiratory depression is evident, monitor arterial blood gases, chest x-ray, and pulmonary function tests.

Treatment Overview:

0.4.2 ORAL EXPOSURE
A) GASTRIC LAVAGE
1) Significant esophageal or gastrointestinal tract irritation or burns may occur following ingestion. The possible benefit of early removal of some ingested material by cautious gastric lavage must be weighed against potential complications of bleeding or perforation.
2) GASTRIC LAVAGE: Consider after ingestion of a potentially life-threatening amount of poison if it can be performed soon after ingestion (generally within 1 hour). Protect airway by placement in Trendelenburg and left lateral decubitus position or by endotracheal intubation. Control any seizures first.
a) CONTRAINDICATIONS: Loss of airway protective reflexes or decreased level of consciousness in unintubated patients; following ingestion of corrosives; hydrocarbons (high aspiration potential); patients at risk of hemorrhage or gastrointestinal perforation; and trivial or non-toxic ingestion.
B) ACTIVATED CHARCOAL
1) Activated charcoal binds most toxic agents and can decrease their systemic absorption if administered soon after ingestion. In general, metals and acids are poorly bound and patients ingesting these materials will not likely benefit from activated charcoal administration.
a) Activated charcoal should not be given to patients ingesting strong acidic or basic caustic chemicals. Activated charcoal is also of unproven value in patients ingesting irritant chemicals, where it may obscure endoscopic findings when the procedure is justified.
2) ACTIVATED CHARCOAL: Administer charcoal as a slurry (240 mL water/30 g charcoal). Usual dose: 25 to 100 g in adults/adolescents, 25 to 50 g in children (1 to 12 years), and 1 g/kg in infants less than 1 year old.
C) DILUTION -
1) Immediate dilution with milk or water may be of benefit in caustic or irritant chemical ingestions.
2) DILUTION: Immediately dilute with 4 to 8 ounces (120 to 240 mL) of water or milk (not to exceed 4 ounces/120 mL in a child).

D) IRRITATION -
1) Observe patients with ingestion carefully for the possible development of esophageal or gastrointestinal tract irritation or burns. If signs or symptoms of esophageal irritation or burns are present, consider endoscopy to determine the extent of injury.
E) OBSERVATION CRITERIA -
1) Carefully observe patients with ingestion exposure for the development of any systemic signs or symptoms and administer symptomatic treatment as necessary.
2) Patients symptomatic following exposure should be observed in a controlled setting until all signs and symptoms have fully resolved.

0.4.3 INHALATION EXPOSURE
A) DECONTAMINATION -
1) INHALATION: Move patient to fresh air. Monitor for respiratory distress. If cough or difficulty breathing develops, evaluate for respiratory tract irritation, bronchitis, or pneumonitis. Administer oxygen and assist ventilation as required. Treat bronchospasm with inhaled beta2 agonist and oral or parenteral corticosteroids.
B) IRRITATION -
1) Respiratory tract irritation, if severe, can progress to pulmonary edema which may be delayed in onset up to 24 to 72 hours after exposure in some cases.
C) ACUTE LUNG INJURY -
1) ACUTE LUNG INJURY: Maintain ventilation and oxygenation and evaluate with frequent arterial blood gas or pulse oximetry monitoring. Early use of PEEP and mechanical ventilation may be needed.
D) BRONCHOSPASM -
1) If bronchospasm and wheezing occur, consider treatment with inhaled sympathomimetic agents.
E) OBSERVATION CRITERIA -
1) Carefully observe patients with inhalation exposure for the development of any systemic signs or symptoms and administer symptomatic treatment as necessary.
2) Patients symptomatic following exposure should be observed in a controlled setting until all signs and symptoms have fully resolved.

0.4.4 EYE EXPOSURE
A) DECONTAMINATION: Irrigate exposed eyes with copious amounts of room temperature water for at least 15 minutes. If irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility.

0.4.5 DERMAL EXPOSURE
A) OVERVIEW
1) DERMAL DECONTAMINATION -
a) DECONTAMINATION: Remove contaminated clothing and wash exposed area thoroughly with soap and water. A physician may need to examine the area if irritation or pain persists.
2) PESTICIDES -
a) DECONTAMINATION: Remove contaminated clothing and jewelry. Wash the skin, including hair and nails, vigorously; do repeated soap washings. Discard contaminated clothing.

3) IRRITATION -
a) Treat dermal irritation or burns with standard topical therapy. Patients developing dermal hypersensitivity reactions may require treatment with systemic or topical corticosteroids or antihistamines.
4) DERMAL ABSORPTION -
a) Some chemicals can produce systemic poisoning by absorption through intact skin. Carefully observe patients with dermal exposure for the development of any systemic signs or symptoms and administer symptomatic treatment as necessary.

Range of Toxicity:

A) No specific range of toxicity can be established for the broad field of chemicals in general.

[Rumack BH POISINDEX(R) Information System Micromedex, Inc., Englewood, CO, 2005; CCIS Volume 125, edition expires Aug, 2005. Hall AH & Rumack BH (Eds): TOMES(R) Information System Micromedex, Inc., Englewood, CO, 2005; CCIS Volume 125, edition expires Aug, 2005., p.]**PEER REVIEWED**

Animal Toxicity Studies:

Non-Human Toxicity Excerpts:

/LABORATORY ANIMALS: Acute Exposure/ /**Ethylenediaminetetraacetic acid**/... produced lowered serum calcium levels, if administered rapidly to animals. Change in serum calcium was small or absent following gradual dosing.
[Hayes, W. J., Jr. Toxicology of Pesticides Baltimore: Williams & Wilkins, 1975., p. 420]**PEER REVIEWED**

/LABORATORY ANIMALS: Acute Exposure/ Large or repeated doses may cause kidney injury. Gastrointestinal upset, pain at the injection site, transient bone marrow depression, mucocutaneous lesions, fever, muscle cramps, and histamine-like reactions (sneezing, lacrimation, nasal congestion) have been reported.
[Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V4 769]**PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ ... Twenty to 40 mg **EDTA** injected during embryogenesis caused tail defects and polydactyly in rat fetuses. ... Cleft palate, brain and eye defects and skeletal anomalies /were produced/ in rat fetuses exposed to 2 or 3% **EDTA** in the diet after day 6 of gestation. By adding 1000 ppm of zinc to the experimental diet prevented the defects.
[Shepard, T.H. Catalog of Teratogenic Agents. 5th ed. Baltimore, MD: The Johns Hopkins University Press, 1986., p. 227]**PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ Congenital defects in the quail were produced with **EDTA**.
[Shepard, T.H. Catalog of Teratogenic Agents. 5th ed. Baltimore, MD: The Johns Hopkins University Press, 1986., p. 227]**PEER REVIEWED**

/GENOTOXICITY/ **EDTA** leads to morphological changes of chromatin and chromosome structure in plant and animal cells. A weak induction of gene mutations has been reported.
[Heindorff K et al; MUTAT RES 115 (2): 149-73 (1983)]**PEER REVIEWED**

/ALTERNATIVE IN VITRO TESTS/ The effect of EDTA (ethylenediaminetetraacetate) on the antimicrobial activity of 10% sodium sulfacetamide solutions was evaluated in this study by kill rate and minimum /inhibitory/ concentration (mic). EDTA improves the kill rate, but not the mic, for the pseudomonas, serratia, and candida species regardless of the preservative.
[Houlsby R et al; J PHARM SCI 72 (12): 1401-3 (1983)]**PEER REVIEWED**

/ALTERNATIVE IN VITRO TESTS/ The effectiveness of EDTA and various chelating agents for releasing glutathione from its cadmium complexes in hemolyzed erythrocytes was studied. EDTA was effective.
[Rabenstein D et al; BIOCHIM BIOPHYS ACTA 762 (4): 531-41 (1983)]**PEER REVIEWED**

/ALTERNATIVE IN VITRO TESTS/ ...Platelets from EDTA platelet-rich plasma (PRP) or CCD PRP to which EDTA has been added do not adhere to glass or plastic surfaces. However, when platelets from EDTA PRP or CCD PRP containing added EDTA are washed and resuspended under conditions reported to cause irreversible dissociation of the fibrinogen receptor, GPIIb/IIIa, then washed and resuspended in buffer containing Ca2+ and Mg2+ ions will adhere and spread in the same manner as platelets not exposed to EDTA.
[White JG et al; Platelets 11 (1): 56-61 (2000)]**PEER REVIEWED**

/ALTERNATIVE IN VITRO TESTS/ ...EDTA demonstrated the highest antifungal activity in comparison with routine antifungal drugs and all other solutions (P<0.0001). Oral cavity isolate was more resistant to the test solutions (P<0.0001).
[Sen BH et al; Oral Surg Oral Med Oral Pathol Oral Radiol Endod 90 (5): 651-5 (2000)]**PEER REVIEWED**

/ALTERNATIVE IN VITRO TESTS/ ...The effect of EDTA /was compared/ with that of EGTA on basal and the agonist (potassium; 8 mM, angiotensin II; 10 nM, ACTH; 10 nM)-stimulated aldosterone production by the cells in vitro. These two chelators lowered the extracellular ionized calcium ([Ca2+]o) concentration in a similar manner. The levels of basal and the agonist-stimulated aldosterone production in the presence of EDTA (1 mM) and EGTA (1 mM) were significantly (P<0.01 or less) increased when compared with those in the absence of EDTA and EGTA, respectively.
[Kigoshi T et al; Endocr J 44 (2): 335-9 (1997)]**PEER REVIEWED**

/OTHER TOXICITY INFORMATION/ ...The lowest dose of EDTA reported to cause a toxic effect in animals was 750 mg/kg/day. ...Oral exposures to EDTA produced adverse reproductive and developmental effects in animals. Clinical tests reported no absorption of an EDTA salt through the skin.
[Lanigan RS et al; Int J Toxicol 21 (Suppl 2): 95-142 (2002)]**PEER REVIEWED**

Ecotoxicity Excerpts:

/AQUATIC SPECIES/ ... Chronic (4 week) exposure to EDTA resulted in increased mortality and reduced growth rates, both of which could be partially ameliorated by adding zinc to culture media.
[Borgmann U, et al; Can J Fish Aquat Sci 52(4): 875-81 (1995)]**PEER REVIEWED**

/AQUATIC SPECIES/ Unicellular alga, poterioochromonas malhamensis, was exposed to inorganic lead or triethyl lead and simultaneously treated with lead antidotes. Some of the antidotes alone slightly to severely inhibited algal growth (bal, EDTA, and salts).
[Roderer G et al; CHEM BIOL INTERACT 46 (2): 247-54 (1983)]**PEER REVIEWED**

National Toxicology Program Studies:

A bioassay of the chelating agent trisodium ethylenediaminetetracetate trihydrate for possible carcinogenicity was conducted by admin the test material in feed to Fischer 344 rats and B6C3F1 mice. The chem was admin to 560 males and 50 females of each species at low and high concn, 3,750 and 7,500 ppm, for 103 wk. Matched control groups were composed of 20 males and 20 females of each species. No cmpd related signs of clinical toxicity were noted. Although a variety of tumors occurred among test and control animals of both species, no tumors were related to treatment. Since survival was satisfactory and showed no consistent variation among test and control groups, the absence of treatment related tumors could not be attributed to early mortality. Levels of Evidence of Carcinogenicity: Male Rats: Negative; Female Rats: Negative; Male Mice: Negative; Female Mice: Negative. /Trisodium ethylenediaminetetraacetate trihydrate/
[Bioassay of Trisodium Ethylenediaminetetraacetate Trihydrate (EDTA) for Possible Carcinogenicity. Technical Rpt Series No. 11 (1977)]**PEER REVIEWED**

Non-Human Toxicity Values:

LD50 Mouse ip 250 mg/kg
[Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 1545]**PEER REVIEWED**

LD50 Rat ip 397 mg/kg
[Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 1545]**PEER REVIEWED**

LD50 Mouse oral 30 mg/kg
[Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V4 769]**PEER REVIEWED**

Ecotoxicity Values:

LC50 Lepomis macrochirus (Bluegill) 159 mg/L/96 hr, pH: 3.7; 96 hr No Observed Effect Concentration: 100 mg/L; static
[Verschueren, K. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. 2001, p. 1099]**PEER REVIEWED**

LC50 Rainbow trout 340 mg/L/24 hr /Conditions of bioassay not specified in source examined/
[Verschueren, K. Handbook of Environmental Data on Organic Chemicals. Volumes 1-2. 4th ed. John Wiley & Sons. New York, NY. 2001, p. 1099]**PEER REVIEWED**

LC50 Lepomis macrochirus (Bluegill, 0.74 (0.62-1.28) g, 34 (28-38) mm) 41 mg/L/96 hr (95% confidence interval: 34-62 mg/L); static /99.33 % a.i., **Versene Acid/**
[Batchelder TL et al; Bull Environ Contam Toxicol 24 (4): 543-549 (1980)]**PEER REVIEWED**

LC50 Ictalurus punctatus (Channel catfish, fingerling 2-3 in) >500 mg/L/1, 2 hr; static /**Versene acid/**
[Clemens HP, Sneed KE; Lethal Doses of Several Commercial Chemicals for Fingerling Channel Catfish. U.S. Fish Wildl Serv Sci Rep Fish No. 316: 10 (1959)]**PEER REVIEWED**

LC50 Ictalurus punctatus (Channel catfish, fingerling 2-3 in) 359 mg/L/4 hr; static /**Versene acid/**
[Clemens HP, Sneed KE; Lethal Doses of Several Commercial Chemicals for Fingerling Channel Catfish. U.S. Fish Wildl Serv Sci Rep Fish No. 316: 10 (1959)]**PEER REVIEWED**

LC50 Ictalurus punctatus (Channel catfish, fingerling 2-3 in) 167 mg/L/24 hr; static /Versene acid/
[Clemens HP, Sneed KE; Lethal Doses of Several Commercial Chemicals for Fingerling Channel Catfish. U.S. Fish Wildl Serv Sci Rep Fish No. 316: 10 (1959)]**PEER REVIEWED**

LC50 Ictalurus punctatus (Channel catfish, fingerling 2-3 in) 133 mg/L/48, 72 hr; static /Versene acid/
[Clemens HP, Sneed KE; Lethal Doses of Several Commercial Chemicals for Fingerling Channel Catfish. U.S. Fish Wildl Serv Sci Rep Fish No. 316: 10 (1959)]**PEER REVIEWED**

LC50 Ictalurus punctatus (Channel catfish, fingerling 2-3 in) 129 mg/L/96 hr; static /Versene acid/
[Clemens HP, Sneed KE; Lethal Doses of Several Commercial Chemicals for Fingerling Channel Catfish. U.S. Fish Wildl Serv Sci Rep Fish No. 316: 10 (1959)]**PEER REVIEWED**

LC50 Daphnia magna (Water flea) 625 mg/L/24 hr; static /formulated product/
[Bringmann G, Kühn R; Wasser-Abwasser-Forsch 10 (5): 161-166 (1977)]**PEER REVIEWED**

LC50 Pimephales promelas (Fathead minnow, 0.3-1 g) 59.8 mg/L/96 hr (95% confidence interval: 44.2-76.5 mg/L/96 hr); static
[Curtis MW, Ward CH; J Hydrol 51: 359-367 (1981)]**PEER REVIEWED**

Metabolism/Pharmacokinetics:

Metabolism/Metabolites:

EDTA is reportedly eliminated essentially unchanged.
[Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V7 769)**PEER REVIEWED**

Absorption, Distribution & Excretion:

EDTA is essentially not metabolized by the human body and it is rapidly excreted in the urine. About 50% of EDTA admin iv is excreted within 1 hr and 90% within 7 hr. EDTA and its metal chelates do not permeate the cellular membrane to a significant extent; thus most of the EDTA remains in the extracellular fluids until excreted into the urine.
[International Labour Office. Encyclopedia of Occupational Health and Safety. Vols. I&II. Geneva, Switzerland: International Labour Office, 1983., p. 443]**PEER REVIEWED**

There is very little metabolic degradation of EDTA The drug is distributed mainly in the extracellular fluids, but very little gains access to the spinal fluid (5%of the plasma concentration).
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1868]**PEER REVIEWED**

Mechanism of Action:

All known pharmacological effects of EDTA result from formation of chelates with divalent and trivalent metal /ions/ in the body.
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001.,

p. 1868]**PEER REVIEWED**

Effects on rat liver glucocorticoid receptor in vitro was studied. At 4 deg C, 10 mmole **EDTA** had a stablizing effect on unbound hepatic glucocorticoid receptors. Apparently, endogenous metal ions are involved in the processes of glucocorticoid-receptor complex stabilization and transformation.
[Hubbard J et al; J STEROID BIOCHEM 19 (2): 1163-7 (1983)]**PEER REVIEWED**

...Editic acid [**ethylenediaminetetraacetic acid (EDTA)**] increases the absorption of some toxicants by increasing intestinal permeability. ...While lead and many other heavy metal ions are not absorbed readily from the GI tract, **EDTA** and other chelators increase the lipid solubility and thus the absorption of complexed ions. Thus, it is important not to give a chelator orally when excess metal is still present in the GI tract after oral ingestion.
[Klaassen, C.D. (ed). Casarett and Doull's Toxicology. The Basic Science of Poisons. 6th ed. New York, NY: McGraw-Hill, 2001., p. 113]**PEER REVIEWED**

Interactions:

Total myocardial calcium content of rats treated with adriamycin was very high. Treatment with **EDTA** decreased calcium levels almost to normal values; however the histological adriamycin-induced cardiac alterations were not prevented.
[Decorti G et al; CANCER LETT 19 (1): 77-83 (1983)]**PEER REVIEWED**

... Increased absorption of drugs ... occurs in presence of ... **ethylenediaminetetraacetic acid (EDTA)**. When given orally ... (100-500 mg/kg in rats), the chelator increased ... /the/ rate of absorption of heparin, sulfopolyglucin, mannitol, inulin, decamethonium, sulfanilic acid ... Phenol red, all lipid-insoluble substances which ordinarily are poorly absorbed from GI tract. The wide variety of the chemical structures of these suggests that the chelating agent is acting in a nonspecific way and is not affecting the physical or chemical state of the compounds within the intestine ... /there is/ direct evidence that **EDTA** acts by increasing the permeability of the intestinal epithelium ... Perhaps **EDTA** alters permeability by increasing the size of the membrane pores or by widening the spaces between the epithelial cells through the removal of calcium ions.
[LaDu, B.N., H.G. Mandel, and E.L. Way. Fundamentals of Drug Metabolism and Disposition. Baltimore: Williams and Wilkins, 1971., p. 35]**PEER REVIEWED**

The effects of **EDTA** on contractile responses of hamster cremaster arterioles and rat aortic strips to epinephrine (epi) or norepinephrine (nor) were examined. Comparable contractile responses were elicited by lower epi or nor concentration in presence than in absence of **EDTA**. Individual responses were maintained in the presence of **EDTA** but rapidly declined if **EDTA** was not present. Apparently, oxidation of epi and nor reduces apparent vascular reactivity and **EDTA** prevents or delays the reduction.
[Maxwell L et al; MICROVASC RES 26 (1): 81-8 (1983)]**PEER REVIEWED**

EDTA influences chromosome breakage by mutagenic agents. When applied in combination with chemical mutagens, **EDTA** enhances mutagen-induced aberration frequencies. This has been demonstrated for many gene loci in drosophila melanogaster, chlamydomonas reinhardi, neurospora crassa and zea mays. **EDTA** interferes with DNA repair processes that take place after exposure to mutagens. In escherichia coli or micrococcus radiodurans as well as in chinese hamster cells, the fast repair component detectable after treatment with ionizing radiation or bleomycin is inhibited by **EDTA**.
[Heindorff K et al; MUTAT RES 115 (2): 149-73 (1983)]**PEER REVIEWED**

...Vanadium toxicity in chicks was alleviated by feeding... **ethylenediaminetetraacetic acid ...**
[National Research Council. Drinking Water and Health. Volume 3. Washington, DC:

National Academy Press, 1980., p. 353]**PEER REVIEWED**

The influence of chelating agents on the toxicity of oxygen at elevated tension was studied in mice. No increase in toxic effects was observed after administration of EDTA.
[Renner G et al; INT J CLIN PHARMACOL, THER TOXICOL 21 (3): 115-7 (1983)]**PEER REVIEWED**

Pharmacology:

Therapeutic Uses:

Anticoagulants; Antidotes; Chelating Agents
[National Library of Medicine's Medical Subject Headings online file (MeSH, 1999)] **PEER REVIEWED**

MEDICATION (VET): Pharmaceutic aid (chelating agent) in treatment of lead and heavy metal poisoning of farm animals.
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 620]**PEER REVIEWED**

Five agents including EDTA were evaluated for their ability to detoxify periodontally involved diseased human tooth root surfaces. Exposure to 15% EDTA partially eliminated the endotoxin.
[Sarbinoff J et al; J PERIODONTOL 54 (2): 77-80 (1983)]**PEER REVIEWED**

Pretreatment of the human teeth with EDTA-strontium chloride at ph 6 decreased the dissolution of enamel by a subsequent acid etching.
[Curzon M et al; CARIES RES 17 (3): 249-52 (1983)]**PEER REVIEWED**

Treatment of chlorosis.
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 467]**PEER REVIEWED**

Use externally in solution or paste to treat skin lesions in chromium-exposed electroplating workers; in endodontic therapy
[International Labour Office. Encyclopedia of Occupational Health and Safety. Vols. I&II. Geneva, Switzerland: International Labour Office, 1983., p. 443]**PEER REVIEWED**

(VET): In dogs, in dissolution therapy of bile duct stones with EDTA solution the pH value had to be shifted to values around 9 and admixture of amino acids and bile salts seemed to improve dissolution conditions. An alkaline milieu of pH 9.5 per se did not cause tissue toxicity.
[Leuscher U et al; Falk Symp 33 (Bile Acids Cholesterol Health Dis): 407-11 (1983)]**PEER REVIEWED**

Drug Warnings:

... direct contact with EDTA may cause dermal sensitization (eczema) or allergic conjunctivitis.
[Gesellschaft Deutscher Chemiker (GDCh) - Advisory Committee on Existing Chemicals of Environmental Relevance (BUA); S. Hirzel Verlag, P.O. Box 10 10 61, 70009 Stuttgart, Germany, 1997. xxi, 223p. Bibl.ref.]**PEER REVIEWED**

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[Renner G et al; INT J CLIN PHARMACOL, THER TOXICOL 21 (3): 115-7 (1983)]**PEER REVIEWED**

Environmental Fate & Exposure:

Environmental Fate/Exposure Summary:

Ethylenediamine tetraacetic acid's production and use in detergents, pharmaceuticals, textile, metal treatment and agricultural industries, analytical chemistry, as a chelating agent and food preservative may result in its release to the environment through various waste streams. If released to air, a vapor pressure of 2X10-12 mm Hg at 25 deg C indicates ethylenediamine tetraacetic acid will exist solely in the particulate phase in the ambient atmosphere. Particulate-phase ethylenediamine tetraacetic acid will be removed from the atmosphere by wet and dry deposition. The potential for direct photolysis may

exist based upon the UV absorption of ethylenediamine tetraacetic acid in surface waters, where it is complexed with iron(III); a half-life of 11.3 minutes was been reported. If released to soil, ethylenediamine tetraacetic acid is expected to have high mobility based upon an estimated Koc of 98. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's Law constant of 7.7X10-16 atm-cu m/mole. The pKa of ethylenediamine tetraacetic acid is 0.26, indicating that this compound will primarily exist in the dissociated form in the environment and anions generally do not adsorb more strongly to organic carbon and clay than their neutral counterparts. If released into water, ethylenediamine tetraacetic acid is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. Since ethylenediamine tetraacetic acid will exist almost entirely in the ionized form at pH values of 5 to 9, volatilization from water surfaces is not expected to be an important fate process. An estimated BCF of 13 suggests the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions. Occupational exposure to ethylenediamine tetraacetic acid may occur through inhalation and dermal contact with this compound at workplaces where ethylenediamine tetraacetic acid is produced or used. Monitoring data indicate that the general population may be exposed to ethylenediamine tetraacetic acid via ingestion of food and drinking water, and dermal contact with this compound and other consumer products containing ethylenediamine tetraacetic acid. Ethylenediamine tetraacetic acid is commonly detected in groundwater, surface water, and drinking water. (SRC)
PEER REVIEWED

Probable Routes of Human Exposure:

NIOSH (NOES Survey 1981-1983) has statistically estimated that 509,055 workers (315,637 of these are female) are potentially exposed to ethylenediamine tetraacetic acid in the US(1). Occupational exposure to ethylenediamine tetraacetic acid may occur through inhalation and dermal contact with this compound at workplaces where ethylenediamine tetraacetic acid is produced or used(SRC). Monitoring data indicate that the general population may be exposed to ethylenediamine tetraacetic acid via ingestion of food and drinking water, and dermal contact with this compound and other consumer products containing ethylenediamine tetraacetic acid(SRC).
[(1) NIOSH; National Occupational Exposure Survey (NOES) (1983)]**PEER REVIEWED**

Average Daily Intake:

The average daily intake of ethylenediamine tetraacetic acid for a man who drinks two liters of water per day would be a maximum of 50 ug. The acceptable daily intake for man is 0 to 2.5 mg/kg(1).
[(1) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992)]**PEER REVIEWED**

Artificial Pollution Sources:

Ethylenediamine tetraacetic acid's production and use in detergents, pharmaceuticals, textile, metal treatment and agricultural industries, analytical chemistry, as a chelating agent and food preservative(1) may result in its release to the environment through various waste streams(SRC).
[(1) Lewis RJ Sr, ed; Hawley's Condensed Chemical Dictionary. 13th ed. NY, NY: John Wiley & Sons, Inc. pp. 467-8 (1997)]**PEER REVIEWED**

Environmental Fate:

TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 98(SRC), determined from a water solubility of 1,000 mg/L(2) and a regression-derived equation(3), indicates that ethylenediamine tetraacetic acid is expected to have high mobility in soil(SRC). Volatilization of ethylenediamine tetraacetic acid from moist soil surfaces is not expected to be an important fate process (SRC) given a Henry's Law constant that is estimated as 7.7X10-16 atm-cu m/mol at 25 deg C(SRC), derived from its vapor pressure, 2X10-12 mm Hg at 25 deg C(4), and water solubility, 1,000 mg/L(2). Ethylenediamine tetraacetic acid is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 2X10-12 mm Hg(4). The biodegradation of ethylenediamine tetraacetic acid is negligible under aerobic conditions unless complexed with a metal or subject to an enriched culture of microorganisms(5).
[(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 4-9 (1990) (4) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis (1989) (5) Kari FG, Giger W; Wat Res 30: 122-134 (1996)]**PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 98(SRC), determined from a water solubility of 1,000 mg/L(2) and a regression-derived equation(3), indicates that ethylenediamine tetraacetic acid is not expected to adsorb to suspended solids and sediment(SRC). Volatilization from water surfaces is not expected(3) based upon a Henry's Law constant that is estimated as 7.7X10-16 atm-cu m/mol at 25 deg C(SRC), derived from its vapor pressure, 2X10-12 mm Hg at 25 deg C(4), and a water solubility, 1,000 mg/L(2). The pKa of ethylenediamine tetraacetic acid is 0.26(5), indicating that this compound will primarily exist in the dissociated form in water. Therefore, this compound is not expected to adsorb more strongly to organic carbon and clay than its neutral counterparts(6). According to a classification scheme(7), an estimated BCF of 13(SRC), using a water solubility of 1,000 mg/L(2) and a regression-derived equation(8), suggests the potential for bioconcentration in aquatic organisms is low(SRC). A photolysis half-life of 11.3 minutes for the 1:1 iron(III) complex of ethylenediamine tetraacetic acid was measured using an aqueous solution, illuminated in a Xenotest 1200 apparatus(10). The biodegradation of ethylenediamine tetraacetic acid is negligible under aerobic conditions unless complexed with a metal or subject to an enriched culture of microorganisms(9).
[(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 4-9, 15-1 to 15-29 (1990) (4) Daubert TE, Danner RP: Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis (1989) (5) Serjeant, EP, Dempsey, B; Ionisation constants of Organic Acids in Aqueous Solution, 567 (1979) (6) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds, Boca Raton, FL: Lewis Publ (2000) (7) Franke C et al; Chemosphere 29: 1501-14 (1994) (8) Meylan WM et al; Environ Toxicol Chem 18: 664-72 (1999) (9) Kari FG, Giger W; Wat Res 30: 122-134 (1996) (10) Svenson A et al; Chemosphere 18: 1805-8 (1989)]**PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), ethylenediamine tetraacetic acid, which has a vapor pressure of 2X10-12 mm Hg at 25 deg C(2), is expected to exist solely in the particulate phase in the ambient atmosphere. Particulate-phase ethylenediamine tetraacetic acid may be removed from the air by wet and dry deposition(SRC).
[(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis (1989)]**PEER REVIEWED**

Environmental Biodegradation:

AEROBIC: In the summer and winter daily cycle of a 5 kg/d load at a Swiss wastewater treatment plant, the concentration of ethylenediamine tetraacetic acid in the primary and secondary effluents was 30-150 ug/L. Therefore, it was concluded that ethylenediamine tetraacetic acid was not biologically degraded. Under nitrifying conditions (sludge age of 12 d and temperature 20 deg C) no biological degradation was observed(1). Another study found that during the waste water treatment process, ethylenediamine tetraacetic acid was not biologically nor chemically degraded in measurable amounts. However, in natural waters and waste water where ethylenediamine tetraacetic acid is complexed with metals, the iron(III) complex is photodegradable and other complexes either degraded slowly or not at all(2). Another article reported a 17-30% reduction of the ethylenediamine tetraacetic acid in three waste water treatment plants in Finland(3). An enriched culture of microorganisms has been found to be an effective method for the degradation of ethylenediamine tetraacetic acid. It may be possible to incorporate this method in wastewater treatment processes to reduce the amount of ethylenediamine tetraacetic acid that is released into the environment(4).
[(1) Alder AC et al; Wat Res 24: 733-742 (1990) (2) Kari FG, Giger W; Wat Res 30: 122-134 (1996) (3) Sillanpaa M; Chemosphere 33: 293-302 (1996) (4) Nortemann B; Appl Microbiol Biotechnol 51: 751-759 (1999)]**PEER REVIEWED**

ANAEROBIC: Ethylenediamine tetraacetic acid was not found to biodegrade in measurable amounts under anaerobic conditions(1).
[(1) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992)]**PEER REVIEWED**

Environmental Abiotic Degradation:

The rate constant for the vapor-phase reaction of ethylenediamine tetraacetic acid with photochemically-produced hydroxyl radicals has been estimated as 1.8X10-10 cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 0.7 hours at an atmospheric concentration of 5X10+5 hydroxyl radicals per cu cm(1). The UV absorption of ethylenediamine tetraacetic acid in surface waters, where it is complexed with iron(III), was found to be 258 nm in an acidic solution and 242 in a basic solution(2). The rate of photolysis of the iron(III) complex of ethylenediamine tetraacetic acid was analyzed. An aqueous solution of the complex was illuminated in a Xenotest 1200 apparatus. Using a sun spectrum from 60 deg N (Stockholm latitude), the half-life was calculated to be 11.3 min for the 1:1 complex dissolved in the top layer of a water body and illuminated at the yearly max of sunlight in the specified area(3).
[(1) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (2) Frank R, Rau H; Ecotoxicol Environ Safety 19: 55-63 (1990) (3) Svenson A et al; Chemosphere 18: 1805-8 (1989)]**PEER REVIEWED**

Ethylenediamine tetraacetic acid (**EDTA**) is capable of complexing with most of the important trace metals and alkaline earth metals in the environment, thus causing an increase in the total solubility of the metals(1). Analysis of cation-**EDTA** equilibria reactions suggests that **EDTA** will eventually predominate as the iron (III) (Fe) chelate in acidic soils and as the calcium (Ca) chelate in alkaline soils (2). **EDTA**, particularly as the Fe(III) chelate, is known to photodegrade(2). The following photodegradation products of Fe(III)-**EDTA** have been identified: carbon monoxide, formaldehyde, ethylenediaminetriacetic acid (ED3A), iminodiacetic acid (IDA), N,N-ethylendiaminediacetic acid (N,N-EDDA), N,N'-EDDA, ethylenediaminediacetic acid (EDMA) and glycine(2,3).
[(1) Gardiner J; Water Res 10: 507 (1976) (2) Tiedje JM; J Environ Qual 6: 21 (1977) (3) Means JL et al; Environ Poll Ser B Chem Phys 1: 45 (1980)]**PEER REVIEWED**

Environmental Bioconcentration:

An estimated BCF of 13 was calculated for ethylenediamine tetraacetic acid(SRC), using a water solubility of 1,000 mg/L(1) and a regression-derived equation(2). According to a classification scheme (3), this BCF suggests the potential for bioconcentration in aquatic organisms is low(SRC).
[(1) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992) (2) Meylan WM et al; Environ Toxicol Chem 18: 664-72 (1999) (3) Franke C et al; Chemosphere 29: 1501-14 (1994)]**PEER REVIEWED**

Soil Adsorption/Mobility:

The Koc of ethylenediamine tetraacetic acid is estimated as 98(SRC), using a water solubility of 1,000 mg/L at 25 deg C(1) and a regression-derived equation(2). According to a classification scheme(3), this estimated Koc value suggests that ethylenediamine tetraacetic acid is expected to have high mobility in soil. The pKa of ethylenediamine tetraacetic acid is 0.26(4), indicating that this compound will primarily exist in the dissociated form in the environment and anions generally do not adsorb more strongly to organic carbon and clay than their neutral counterparts(5).
[(1) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 4-9 (1990) (3) Swann RL et al; Res Rev 85: 17-28 (1983) (4) Serjeant EP, Dempsey B; Ionisation constants of Organic Acids in Aqueous Solution. NY, NY: Pergamon p. 567 (1979) (5) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000)]**PEER REVIEWED**

Volatilization from Water/Soil:

The Henry's Law constant for ethylenediamine tetraacetic acid is estimated as 7.7X10-16 atm-cu m/mol at 25 deg C(SRC) derived from its vapor pressure, 2X10-12 mm Hg mm Hg(1), and water solubility, 1,000 mg/l(2). This Henry's Law constant indicates that ethylenediamine tetraacetic acid is expected to be essentially nonvolatile from water surfaces(3). Ethylenediamine tetraacetic acid's estimated Henry's Law constant indicates that volatilization from moist soil surfaces may occur(SRC). Ethylenediamine tetraacetic acid is not expected to volatilize from dry soil surfaces(SRC) based upon its vapor pressure of 2X10-12 mm Hg(1).
[(1) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, DC: Taylor and Francis (1989) (2) Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990)]**PEER REVIEWED**

Environmental Water Concentrations:

GROUNDWATER: Ethylenediamine tetraacetic acid was detected in ground water samples at concentrations ranging from 5 to 25 ug/L, location not specified(1).
[(1) Alder AC et al; Water Res 24: 733-42 (1990)]**PEER REVIEWED**

DRINKING WATER: Ethylenediamine tetraacetic acid was detected in drinking water produced from surface waters in concentrations ranging from 10 to 45 ug/L(1), location not specified. Ethylenediamine tetraacetic acid was detected in the drinking water of Dutch drinking water companies in March 1992 at concentrations ranging from <0.8 to 16.5 ug/L(2).

[(1) Alder AC et al; Water Res 24: 733-42 (1990) (2) Bergers PJM, de Groot AC; Water Res 28: 639-42 (1994)]**PEER REVIEWED**

SURFACE WATER: During 1974, ethylenediamine tetraacetic acid (EDTA) was identified in the Lea River (England) at concentrations ranging from approximately zero to 1,120 ppb(1). Ethylenediamine tetraacetic acid was detected in the surface water of Dutch drinking water companies in March 1992 at concentrations ranging from 2.6 to 29.2 ug/L(2).
[(1) Gardiner J; Water Res 10: 507 (1976) (2) Bergers PJM, de Groot AC; Water Res 28: 639-42 (1994)]**PEER REVIEWED**

Effluent Concentrations:

During 1974, ethylenediamine tetraacetic acid (EDTA) was identified in the effluent from the Rye Meads (England) sewage treatment plant at concentrations ranging from 200 ppb to 1200 ppb(1). The concentration of ethylenediamine tetraacetic acid waste water effluents of five Finnish pulp and paper mills was between 0.24 mg/L and 8.4 mg/L(2).
[(1) Gardiner J; Water Res 10: 507 (1976) (2) Sillanpaa M; Chemosphere 33: 293-302 (1996)]**PEER REVIEWED**

Sediment/Soil Concentrations:

SEDIMENT: Ethylenediamine tetraacetic acid was detected in lake sediment samples collected from Lake Saimaa in Finland during May and June of 1996 at concentrations ranging from 80 to 310 ug/kg (1). The fraction of ethylenediamine tetraacetic acid absorbed onto sediment in samples of surface waters, groundwaters, and waste water treatment plant effluents from Switzerland was an average of one percent(2).
[(1) Sillanpaa M et al; Chemosphere 35: 2797-805 (1997) (2) Nowack B et al; Anal Chem 68: 561-66 (1996)]**PEER REVIEWED**

Environmental Standards & Regulations:

FIFRA Requirements:

As the federal pesticide law FIFRA directs, EPA is conducting a comprehensive review of older pesticides to consider their health and environmental effects and make decisions about their future use. Under this pesticide reregistration program, EPA examines health and safety data for pesticide active ingredients initially registered before November 1, 1984, and determines whether they are eligible for reregistration. In addition, all pesticides must meet the new safety standard of the Food Quality Protection Act of 1996. Pesticides for which EPA had not issued Registration Standards prior to the effective date of FIFRA '88 were divided into three lists based upon their potential for human exposure and other factors, with List B containing pesticides of greater concern and List D pesticides of less concern. **Ethylenediaminetetraacetic acid** is found on List D. Case No: 4036; Pesticide type: insecticide fungicide, herbicide, antimicrobial; Case Status: None of the active ingredients in the case are being supported for reregistration by their registrants. All are unsupported, or some are unsupported and some are cancelled. Cases described as "unsupported" generally are being processed for cancellation.; Active ingredient (AI): **ethylenediaminetetraacetic acid;** AI Status: The active ingredient is no longer contained in any registered products ... "cancelled."
[United States Environmental Protection Agency/ Prevention, Pesticides and Toxic Substances; Status of Pesticides in Registration, Reregistration, and Special Review. (1998) EPA 738-R-98-002, p. 309]**PEER REVIEWED**

Acceptable Daily Intakes:

FAO/WHO ACCEPTABLE DAILY INTAKE: 0-2.5 MG/KG BODY WT.
[Doull, J., C.D. Klaassen, and M. D. Amdur (eds.). Casarett and Doull's Toxicology. 2nd ed. New York: Macmillan Publishing Co., 1980., p. 600]**PEER REVIEWED**

CERCLA Reportable Quantities:

Persons in charge of vessels or facilities are required to notify the National Response Center (NRC) immediately, when there is a release of this designated hazardous substance, in an amount equal to or greater than its reportable quantity of 5000 lb or 2270 kg. The toll free number of the NRC is (800) 424-8802; in the Washington D.C. metropolitan area (202) 426-2675. The rule for determining when notification is required is stated in 40 CFR 302.4 (section IV. D.3.b).
[40 CFR 302.4; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from: http://www.gpoaccess.gov/ecfr as of February 10, 2004]**PEER REVIEWED**

Clean Water Act Requirements:

Ethylenediamine tetraacetic acid is designated as a hazardous substance under section 311(b)(2)(A) of the Federal Water Pollution Control Act and further regulated by the Clean Water Act Amendments of 1977 and 1978. These regulations apply to discharges of this substance. This designation includes any isomers and hydrates, as well as any solutions and mixtures containing this substance.
[40 CFR 116.4; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from: http://www.gpoaccess.gov/ecfr as of February 10, 2004]**QC REVIEWED**

Chemical/Physical Properties:

Molecular Formula:

C10-H16-N2-O8
PEER REVIEWED

Molecular Weight:

292.25
[Lide, DR (ed.). CRC Handbook of Chemistry and Physics. 81st Edition. CRC Press LLC, Boca Raton: FL 2000, p. 3-174]**PEER REVIEWED**

Color/Form:

Crystals from water
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 620]**PEER REVIEWED**

Colorless crystals
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 467]**PEER REVIEWED**

Melting Point:

245 deg C

[Lide, DR (ed.). CRC Handbook of Chemistry and Physics. 81st Edition. CRC Press LLC, Boca Raton: FL 2000, p. 3-174]**PEER REVIEWED**

Dissociation Constants:

pKa = 0.26
[Serjeant, E.P., Dempsey B.; Ionisation Constants of Organic Acids in Aqueous Solution. International Union of Pure and Applied Chemistry (IUPAC). IUPAC Chemical Data Series No. 23, 1979. New York, New York: Pergamon Press, Inc., p. 567]**PEER REVIEWED**

Octanol/Water Partition Coefficient:

log Kow = -3.86 /Estimated/
[US EPA; Estimation Program Interface (EPI) Suite. Ver.3.11. June 10, 2003. Available at http://www.epa.gov/oppt/exposure/docs/episuitedl.htmas of Feb 12, 2004.]**PEER REVIEWED**

Solubilities:

SOL IN SOLN OF ALKALI HYDROXIDES
[Osol, A. (ed.). Remington's Pharmaceutical Sciences. 16th ed. Easton, Pennsylvania: Mack Publishing Co., 1980., p. 1260]**PEER REVIEWED**

Insol in common organic solvents
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 467]**PEER REVIEWED**

In water, 1,000 mg/L @ 25 deg C
[Wolf K, Gilbert PA; in The Handbook of Environmental Chemistry. Hutzinger O, ed. Berlin, Germany: Spring-Verlag 3F: 243-259 (1992)]**PEER REVIEWED**

Spectral Properties:

IR: 230F (Aldrich Chemical Co., Inc., New York; Aldrich Library of Infrared Spectra, Aldrich Chemical Co., Milwaukee, WI)
[Lide, D.R., G.W.A. Milne (eds.). Handbook of Data on Organic Compounds. Volume I. 3rd ed. CRC Press, Inc. Boca Raton ,FL. 1994., p. V3: 2905]**PEER REVIEWED**

NMR: 3:2D (Aldrich Library of Mass Spectra, Aldrich Chemical Co, Milwaukee, WI)
[Weast, R.C. and M.J. Astle. CRC Handbook of Data on Organic Compounds. Volumes I and II. Boca Raton, FL: CRC Press Inc. 1985., p. V1 622]**PEER REVIEWED**

Vapor Pressure:

2X10-12 mm Hg @ 25 deg C /Extrapolated/
[Daubert, T.E., R.P. Danner. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis, 1989., p.]**PEER REVIEWED**

Other Chemical/Physical Properties:

Decomposition at 220 deg C

[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 620]**PEER REVIEWED**

Neutralized by alkali metal hydroxides to form a series of water-soluble salts containing from one to four alkali metal cations.
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 467]**PEER REVIEWED**

Decomposition at 240 deg C
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 467]**PEER REVIEWED**

Henry's Law constant = 7.69X10-16 atm-cu m/mol @ 25 deg C /Estimated/
[US EPA; Estimation Program Interface (EPI) Suite. Ver.3.11. June 10, 2003. Available at http://www.epa.gov/oppt/exposure/docs/episuitedl.htm as of Feb 12, 2004.]**PEER REVIEWED**

Hydroxyl radical reaction rate constant = 1.82X10-10 cu cm/molc sec @ 25 deg C /Estimated/
[US EPA; Estimation Program Interface (EPI) Suite. Ver.3.11. June 10, 2003. Available at http://www.epa.gov/oppt/exposure/docs/episuitedl.htm as of Feb 12, 2004.]**PEER REVIEWED**

Chemical Safety & Handling:

DOT Emergency Guidelines:

Fire or explosion: Some may burn but none ignite readily. Those substance designated with a "P" may polymerize explosively when heated or involved in a fire. Containers may explode when heated. Some may be transported hot.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

Health: Inhalation of material may be harmful. Contact may cause burns to skin and eyes. Inhalation of asbestos dust may have a damaging effect on the lungs. Fire may produce irritating, corrosive and/or toxic gases. Runoff from fire control may cause pollution.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

Public safety: CALL Emergency Response Telephone Number. ... Isolate spill or leak area immediately for at least 10 to 25 meters (30 to 80 feet) in all directions. Keep unauthorized personnel away. Stay upwind.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

Protective clothing: Wear positive pressure self-contained breathing apparatus (SCBA). Structural firefighters' protective clothing will only provide limited protection.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

Evacuation: Fire: If tank, rail car or tank truck is involved in a fire, ISOLATE for 800 meters (1/2 mile) in all directions; also, consider initial evacuation for 800 meters (1/2 mile) in all directions.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

Fire: Small fires: Dry chemical, CO2, water spray or regular foam. Large fires: Water spray, fog or regular foam. Move containers from fire area if you can do it without risk. Do not scatter spilled material with high pressure water streams. Dike fire-control water for later disposal. Fire involving tanks: Cool containers with flooding quantities of water until well after fire is out. Withdraw immediately in case of rising sound from venting safety devices or discoloration of tank. ALWAYS stay away from engulfed in fire tanks.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

Spill or leak: Do not touch or walk through spilled material. Stop leak if you can do it without risk. Prevent dust cloud. Avoid inhalation of asbestos dust. Small dry spills: With clean shovel place material into clean, dry container and cover loosely: move containers from spill area. Small spills: Take up with sand or other noncombustible absorbent material and place into containers for later disposal. Large spills: Dike far ahead of liquid spill for later disposal. Cover powder spill with plastic sheet or tarp to minimize spreading. Prevent entry into waterways, sewers, basements or confined areas.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

First aid: Move victim to fresh air. Call 911 or emergency medical service. Apply artificial respiration if victim is not breathing. Administer oxygen if breathing is difficult. Remove and isolate contaminated clothing and shoes. In case of contact with substance, immediately flush skin or eyes with running water for at least 20 minutes. Ensure that medical personnel are aware of the material(s) involved, and take precautions to protect themselves.
[U.S. Department of Transportation. 2000 Emergency Response Guidebook. RSPA P 5800.8 Edition. Washington, D.C: U.S. Government Printing Office, 2000,p. G-171] **PEER REVIEWED**

Fire Fighting Procedures:

If material involved in fire: Extinguish fire using agent suitable for type of surrounding fire. (Material itself does not burn or burns with difficulty.)
[Association of American Railroads/Bureau of Explosives; Emergency Handling of Hazardous Materials in Surface Transportation. Association of American Railroads. Pueblo, CO. 2002., p. 425]**PEER REVIEWED**

Hazardous Reactivities & Incompatibilities:

Reacts violently with lead dioxide.
[International Labour Office. Encyclopaedia of Occupational Health and Safety. 4th edition, Volumes 1-4 1998. Geneva, Switzerland: International Labour Office, 1998., p. 104.124]**PEER REVIEWED**

Reacts with oxidants.
[International Labour Office. Encyclopaedia of Occupational Health and Safety. 4th edition, Volumes 1-4 1998. Geneva, Switzerland: International Labour Office, 1998.,

p. 104.124]**PEER REVIEWED**

Hazardous Decomposition:

When heated to decomp it emits toxic fume of /nitrogen oxides/.
[Lewis, R.J. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 1545]**PEER REVIEWED**

Protective Equipment & Clothing:

Personnel protection: ... Wear appropriate chemical protective gloves, boots and goggles. ...
[Association of American Railroads/Bureau of Explosives; Emergency Handling of Hazardous Materials in Surface Transportation. Association of American Railroads. Pueblo, CO. 2002., p. 426]**PEER REVIEWED**

Preventive Measures:

Personnel protection: Keep upwind. ... Avoid breathing vapors or dusts. Wash away any material which may have contacted the body with copious amounts of water or soap and water.
[Association of American Railroads/Bureau of Explosives; Emergency Handling of Hazardous Materials in Surface Transportation. Association of American Railroads. Pueblo, CO. 2002., p. 426]**PEER REVIEWED**

If material not involved in fire: Keep material out of water sources and sewers. Build dikes to contain flow as necessary.
[Association of American Railroads/Bureau of Explosives; Emergency Handling of Hazardous Materials in Surface Transportation. Association of American Railroads. Pueblo, CO. 2002., p. 426]**PEER REVIEWED**

Stability/Shelf Life:

The free acid is less stable than its salts, and tends to decarboxylate when heated to temps of 150 deg C. Stable on storage and on boiling in aq solution.
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 620]**PEER REVIEWED**

Cleanup Methods:

Environmental considerations: Land spill: Dig a pit, pond, lagoon, holding area to contain liquid or solid material. /SRP: If time permits, pits, ponds, lagoons, soak holes, or holding areas should be sealed with an impermeable flexible membrane liner./ Cover solids with a plastic sheet to prevent dissolving in rain or fire fighting water.
[Association of American Railroads/Bureau of Explosives; Emergency Handling of Hazardous Materials in Surface Transportation. Association of American Railroads. Pueblo, CO. 2002., p. 426]**PEER REVIEWED**

Environmental considerations: Water spill: Add dilute caustic soda (NaOH). Adjust pH to neutral (pH). Use mechanical dredges or lifts to remove immobilized masses of pollutants and precipitates.
[Association of American Railroads/Bureau of Explosives; Emergency Handling of Hazardous Materials in Surface Transportation. Association of American Railroads. Pueblo, CO. 2002., p. 426]**PEER REVIEWED**

Disposal Methods:

SRP: The most favorable course of action is to use an alternative chemical product with less inherent propensity for occupational exposure or environmental contamination. Recycle any unused portion of the material for its approved use or return it to the manufacturer or supplier. Ultimate disposal of the chemical must consider: the material's impact on air quality; potential migration in soil or water; effects on animal, aquatic, and plant life; and conformance with environmental and public health regulations.
[ ]**PEER REVIEWED**

Occupational Exposure Standards:

Manufacturing/Use Information:

Major Uses:

The active ingredient is no longer contained in any registered products ... "cancelled."
[United States Environmental Protection Agency/ Prevention, Pesticides and Toxic Substances; Status of Pesticides in Registration, Reregistration, and Special Review. (1998) EPA 738-R-98-002, p. 309]**PEER REVIEWED**

For **ethylenediaminetetraacetic acid** (USEPA/OPP Pesticide Code: 039101) there are 0 labels match. /SRP: Not registered for current use in the U.S., but approved pesticide uses may change periodically and so federal, state and local authorities must be consulted for currently approved uses./
[U.S. Environmental Protection Agency/Office of Pesticide Program's Chemical Ingredients Database on Ethylenediaminetetraacetic Acid (60-00-4). Available from the Database Query page at http://www.cdpr.ca.gov/docs/epa/epamenu.htm as of February 10, 2004]**PEER REVIEWED**

As antioxidant in foods
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 621]**PEER REVIEWED**

Chelating agent in boiler and cooling water, detergents for household and textile use, industrial germicides, and metal cutting fluids; chemical intermediate for micronutrients for agricultural purposes; bleaching agent in color film processing; chelating agent in electroless nickel plating; etching agent in metal finishing and semiconductor production; chelating agent in wood pulping processes; activator in butadiene-styrene copolymerization; chelating agent in food, pharmaceuticals and cosmetics and for gas scrubbing; component of blood anticoagulants
[SRI, p.]**PEER REVIEWED**

MEDICATION
PEER REVIEWED

MEDICATION (VET)
PEER REVIEWED

Liq soaps, shampoos, agricultural chem sprays; metal plating, decontamination of radioactive surfaces, metal deactivator in vegetable oil, oil emulsions, pharmaceutical products, etc; eluting agent in ion exchange, to remove insoluble deposits of calcium & magnesium soaps; in textiles to improve dyeing, scouring; clarification of liq, analitical chemistry, spectrophotometric titration, aid in reducing blood cholesterol, food additive (preservative)

[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 467]**PEER REVIEWED**

Is effective in detaching biofilm
[National Research Council. Drinking Water & Health, Volume 4. Washington, DC: National Academy Press, 1981., p. 117]**PEER REVIEWED**

Ethylenediaminetetraacetic acid (EDTA, edathamil), its sodium salt (disodium edetate, Na2EDTA), and ... closely related compounds have been used as industrial and analytical reagents because they chelate many divalent & trivalent metals.
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1867]**PEER REVIEWED**

... **EDTA** ... aids in the flavor retention of stored beverages and ... has been shown effective in controlling corrosion in canned carbonated beverages. ... Experimentally, off-flavor /in whole milk/ has been shown to be suppressed by adding to milk at least 5 parts of **EDTA** per part copper. ... **EDTA** enhances the foaming properties of reconstituted skim milk. ... **EDTA** will retard nitric oxide-hemoglobin formation in cured meats by controlling the effects of metal ions.
[Furia, T.E. (ed.). CRC Handbook of Food Additives. 2nd ed. Cleveland: The Chemical Rubber Co., 1972., p. 286]**PEER REVIEWED**

Most of the official drugs containing calcium and the zinc content of zinc stearate are analyzed by titration with a standard **EDTA** solution.
[Osol, A. (ed.). Remington's Pharmaceutical Sciences. 16th ed. Easton, Pennsylvania: Mack Publishing Co., 1980., p. 183]**PEER REVIEWED**

Manufacturers:

Dow Chemical U.S.A., 2030 Dow Center, Midland MI 48642, (989) 636-1000; Production site: Freeport, TX 77541
[SRI Consulting. 2003 Directory of Chemical Producers. SRI International, Menlo Park, CA. 2003, p. 506]**PEER REVIEWED**

Hampshire Chemical Corp., 45 Hayden Ave., Ste 2500, Lexington, MA 02421-7994, (781) 869-3400; Production sites: Deer Park, TX 77536; Nashua, NH 03060
[SRI Consulting. 2003 Directory of Chemical Producers. SRI International, Menlo Park, CA. 2003, p. 506]**PEER REVIEWED**

Methods of Manufacturing:

Ethylenediamine is condensed with sodium monochloroacetate with aid of sodium carbonate. Aqueous solution of reactants is heated to about 90 deg C for 10 hr, then cooled and acidified with hydrochloric acid whereupon the acid precipitates. Salts of acid are known as edetates.
[Osol, A. (ed.). Remington's Pharmaceutical Sciences. 16th ed. Easton, Pennsylvania: Mack Publishing Co., 1980., p. 1260]**PEER REVIEWED**

Heating tetrahydroxyethylethylenediamine with sodium hydroxide or potassium hydroxide with calcium oxide catalyst.
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc. 1997., p. 467]**PEER REVIEWED**

The most widely used synthesis is the alkaline cyanomethylation of ethylenediamine by means of

sodium cyanide and formaldehyde.
[Ullmann's Encyclopedia of Industrial Chemistry. 6th ed.Vol 1: Federal Republic of Germany: Wiley-VCH Verlag GmbH & Co. 2003 to Present, p. V12 587 (2003)]**PEER REVIEWED**

... Commercial method for production of EDTA is the two-step Singer synthesis. In this process, the cyanomethylation step is separate from the hydrolysis. Hydrogen cyanide and formaldehyde react with ethylenediamine to form insoluble (ethylenedinitrilo)tetraacetonitrile (EDTN), 2,2',2",2"'-(1,2-ethanediyldinitrilo)tetrakis(acetonitrile), in high yield (>96%). The intermediate nitrile is separated, washed, and subsequently hydrolyzed with sodium hydroxide to tetrasodium EDTA, with liberation of byproduct ammonia. Carrying out the synthesis in two stages eliminates most of the impurity-forming reactions and yields a very pure form of chelating agent.
[Ullmann's Encyclopedia of Industrial Chemistry. 6th ed.Vol 1: Federal Republic of Germany: Wiley-VCH Verlag GmbH & Co. 2003 to Present, p. V12 587 (2003)]**PEER REVIEWED**

General Manufacturing Information:

Addition of EDTA to contact lens storing and rinsing solution prevents the inactivation of the protein remover which is present in the formulation.
[LUDWIG G, SUNDERDICK R; CAN PATENT NUMBER 1150907 8/2/83 (TIMUS EUROCON KONTAKTLINSEN GMBH UND CO, K-G)]**PEER REVIEWED**

The vase-life of cut flowers stored at low temp was extended by treating the flowers, especially carnations, before storing with solution containing EDTA, silver nitrate, sodium thiosulfate, and sucrose.
[RUDNICKI RM ET AL; POL PATENT NUMBER 2/25/83 (INSTYTUT SADOWNICTWA, SKIERNIEWICE)]**PEER REVIEWED**

The concentration of EDTA-tris used as lavage to treat otitis externa, cystitis or other persistent infections in dogs and cats, was found to prevent the growth of Pseudomonas aeruginosa, Staphylococcus aureus and beta Streptococci when present in growth media.
[Wooley RE, Jones MS; Vet Microbiol 8 (3): 271-80 (1983)]**PEER REVIEWED**

Edetic acid has increased antibacterial effects of benzalkonium and chlorocresol, as well as preventing resistance in vitro to neomycin by resistant strains of Staphylococcus aureus. Copper, manganese, zinc utilization in chicks is enhanced by prior chelation with **edetic acid.**
[Rossoff, I.S. Handbook of Veterinary Drugs. New York: Springer Publishing Company, 1974., p. 193]**PEER REVIEWED**

Acid, rather than any salt, is form most potent in removing calcium from solution. It may be added to shed blood to prevent clotting. It is also used in pharmaceutical analysis and removal or inactivation of unwanted ions in solution.
[Osol, A. (ed.). Remington's Pharmaceutical Sciences. 16th ed. Easton, Pennsylvania: Mack Publishing Co., 1980., p. 1260]**PEER REVIEWED**

/Ethylenediaminetetraacetic acid/ was used as analytical reagent and as chemical in manufacture of dyes as early as 1935.
[Hayes, W. J., Jr. Toxicology of Pesticides Baltimore: Williams & Wilkins, 1975., p. 420]**PEER REVIEWED**

Essentially 100% as a chelating agent (includes free acid plus salts)
[SRI, p.]**PEER REVIEWED**

U. S. Production:

(1979) 4.49X10+9 G
[SRI, p.]**PEER REVIEWED**

(1982) 2.82X10+9 G
[SRI, p.]**PEER REVIEWED**

(1985) 4.49X10+9 g
[USITC, SYN ORG CHEM-US PROD/SALES 1985 p.245]**PEER REVIEWED**

U. S. Imports:

(1978) ND
[SRI, p.]**PEER REVIEWED**

(1982) ND
[SRI, p.]**PEER REVIEWED**

(1987) ND
PEER REVIEWED

U. S. Exports:

(1978) ND
[SRI, p.]**PEER REVIEWED**

(1982) ND
[SRI, p.]**PEER REVIEWED**

(1987) ND
PEER REVIEWED

Laboratory Methods:

Analytic Laboratory Methods:

EDTA in pickled vegetables was identified by gas-liquid chromatography and confirmed by mass fragmentography after conversion to its tetramethyl ester.
[WILLIAMS DT; J ASSOC OFF ANAL CHEM 57 (6): 1382-85 (1974)]**PEER REVIEWED**

A spectrophotometric method for determining **EDTA** in freshwater is presented. The sensitivity of the method is 10 ug.
[KAISER K LE; WATER RES 7 (10): 1465-73 (1973)]**PEER REVIEWED**

Ethylenediaminetetraacetic acid and related chelating agents are readily analyzed by titration with standardized solutions of metal salts, which react quantitatively to form metal chelate complexes. The endpoint is commonly determined by the precipitation and turbidity of an insoluble metal compound or the color change of a metal-sensitive dye used as an indicator.
[Ullmann's Encyclopedia of Industrial Chemistry. 6th ed.Vol 1: Federal Republic of Germany: Wiley-VCH Verlag GmbH & Co. 2003 to Present, p. V12 588 (2003)]**PEER REVIEWED**

Method: ASTM D3113A, Standard Test Methods for Sodium Salts of EDTA in Water; Analyte: **ethylenediaminetetraacetic acid;** Matrix: Waters containing free Na4EDTA or heavy metal or alkaline earth chelates of Na4EDTA either individually or in combination, in concentrations from 0.5 to 20 mg/L. Higher concentrations may be determined by dilution; Detection Level: 0.5 mg/L.
[National Environmental Methods Index; Analytical, Test and Sampling Methods. Available from http://infotrek.er.usgs.gov/servlet/page?_pageid=202,204,1160&_dad=portal30&_schema=PORTAL30 on Ethylenediaminetetraacetic Acid (60-00-4) as of February 10, 2004]**PEER REVIEWED**

Method: ASTM D3113B, Standard Test Methods for Sodium Salts of EDTA in Water; Analyte: **ethylenediaminetetraacetic acid;** Matrix: Waters containing unchelated EDTA in concentrations of 1 to 50 mg/L. Higher concentrations may be determined by diluting the sample; Detection Level: 1 mg/L.
[National Environmental Methods Index; Analytical, Test and Sampling Methods. Available from http://infotrek.er.usgs.gov/servlet/page?_pageid=202,204,1160&_dad=portal30&_schema=PORTAL30 on Ethylenediaminetetraacetic Acid (60-00-4) as of February 10, 2004]**PEER REVIEWED**

Special References:

Special Reports:

HART JR; COSMET TOILETRIES 98 (4): 54-8 (1983); USE OF EDTA-TYPE CHELATING AGENTS & THEIR SALTS IN SHAMPOOS, LIQUID SOAPS, SKIN CLEANERS, CREAMS & LOTIONS, & OTHER PRODUCTS ARE REVIEWED.

NTP TR No 011; Route: oral in feed; Species: rats and mice. NTIS No PB270938/AS. /Trisodium ethylenediaminetetraacetate trihydrate/
[NTP; Division of Toxicology Research and Testing; Management Status Report; 07/07/93; p.28]

Synonyms and Identifiers:

Related HSDB Records:

4072 [DISODIUM CALCIUM **EDTA**] (salt)

5003 [TETRASODIUM **EDTA**] (salt)

Synonyms:

ACETIC ACID, 2,2',2",2"'-(1,2-ETHANEDIYLDINITRILO)TETRAKIS-
PEER REVIEWED

ACETIC ACID, (ETHYLENEDINITRILO)TETRA-
PEER REVIEWED

ACIDE ETHYLENEDIAMINETETRACETIQUE (FRENCH)
PEER REVIEWED

AI3-17181
PEER REVIEWED

Caswell No 438
PEER REVIEWED

CELON A
PEER REVIEWED

CELON ATH
PEER REVIEWED

CHEELOX
PEER REVIEWED

CHEELOX BF ACID
PEER REVIEWED

CHEMCOLOX 340
PEER REVIEWED

COMPLEXON II
PEER REVIEWED

3,6-DIAZAOCTANEDIOIC ACID, 3,6-BIS(CARBOXYMETHYL)-
PEER REVIEWED

EDATHAMIL
PEER REVIEWED

EDETIC
PEER REVIEWED

EDETIC ACID
PEER REVIEWED

EDTA
PEER REVIEWED

EDTA ACID
PEER REVIEWED

ENDRATE
PEER REVIEWED

EPA Pesticide Chemical Code 039101
PEER REVIEWED

N,N'-1,2-ETHANEDIYLBIS[N-(CARBOXYMETHYL)GLYCINE]
PEER REVIEWED

Ethylenebisiminodiacetic Acid
PEER REVIEWED

ETHYLENEDIAMINETETRAACETIC ACID

PEER REVIEWED

ETHYLENEDIAMINE-N,N,N',N'-TETRAACETIC ACID
PEER REVIEWED

GLYCINE, N,N'-1,2-ETHANEDIYLBIS(N-(CARBOXYMETHYL)-
PEER REVIEWED

HAVIDOTE
PEER REVIEWED

METAQUEST A
PEER REVIEWED

NERVANAID B ACID
PEER REVIEWED

NULLAPON BF ACID
PEER REVIEWED

PERMA KLEER 50 ACID
PEER REVIEWED

SEQUESTRENE AA
PEER REVIEWED

SEQUESTRIC ACID
PEER REVIEWED

SEQUESTROL
PEER REVIEWED

TETRINE ACID
PEER REVIEWED

TITRIPLEX
PEER REVIEWED

TRICON BW
PEER REVIEWED

TRILON BW
PEER REVIEWED

VERSENE
PEER REVIEWED

VERSENE ACID
PEER REVIEWED

VINKEIL 100
PEER REVIEWED

WARKEELATE ACID
PEER REVIEWED

(Ethylenedinitrilo)tetraacetic acid
PEER REVIEWED

USEPA/OPP Pesticide Code: 039101
PEER REVIEWED

Associated Chemicals:

Trisodium Ethylenediaminetetraacetate trihydrate;150-38-9

Shipping Name/ Number DOT/UN/NA/IMO:

NA 9117; Ethylenediaminetetraacetic acid; EDTA

Standard Transportation Number:

49 669 10; Ethylenediaminetetraacetic acid

Administrative Information:

Hazardous Substances Databank Number: 809

Last Revision Date: 20040910

Last Review Date: Reviewed by SRP on 5/13/2004

Update History:

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Complete Update on 02/14/2003, 1 field added/edited/deleted.
Complete Update on 11/08/2002, 1 field added/edited/deleted.
Complete Update on 10/16/2002, 2 fields added/edited/deleted.
Field Update on 08/06/2002, 1 field added/edited/deleted.
Field Update on 01/14/2002, 1 field added/edited/deleted.
Field Update on 08/08/2001, 1 field added/edited/deleted.
Field Update on 05/15/2001, 1 field added/edited/deleted.
Complete Update on 06/12/2000, 1 field added/edited/deleted.
Complete Update on 02/08/2000, 1 field added/edited/deleted.
Complete Update on 02/02/2000, 1 field added/edited/deleted.
Complete Update on 11/18/1999, 1 field added/edited/deleted.
Complete Update on 09/21/1999, 1 field added/edited/deleted.
Complete Update on 08/26/1999, 1 field added/edited/deleted.
Complete Update on 06/03/1999, 1 field added/edited/deleted.
Complete Update on 01/27/1999, 1 field added/edited/deleted.
Complete Update on 06/02/1998, 1 field added/edited/deleted.
Complete Update on 02/27/1998, 1 field added/edited/deleted.
Complete Update on 10/17/1997, 1 field added/edited/deleted.

Complete Update on 09/16/1997, 2 fields added/edited/deleted.
Complete Update on 04/01/1997, 2 fields added/edited/deleted.
Complete Update on 03/11/1997, 2 fields added/edited/deleted.
Complete Update on 02/20/1997, 1 field added/edited/deleted.
Complete Update on 01/24/1997, 1 field added/edited/deleted.
Complete Update on 09/10/1996, 1 field added/edited/deleted.
Complete Update on 05/10/1996, 1 field added/edited/deleted.
Complete Update on 01/19/1996, 1 field added/edited/deleted.
Complete Update on 12/22/1994, 1 field added/edited/deleted.
Complete Update on 05/05/1994, 1 field added/edited/deleted.
Complete Update on 03/25/1994, 1 field added/edited/deleted.
Complete Update on 09/16/1993, 1 field added/edited/deleted.
Field update on 12/15/1992, 1 field added/edited/deleted.
Complete Update on 09/26/1991, 1 field added/edited/deleted.
Complete Update on 10/22/1990, 7 fields added/edited/deleted.
Complete Update on 04/13/1989, 1 field added/edited/deleted.
Complete Update on 12/28/1988, 57 fields added/edited/deleted.
Complete Update on 10/03/1986
Created 19830401 by GCF

United States Patent **5,437,870**
Puritch , et al. **August 1, 1995**

Ingestible mollusc poisons

Abstract

An effective, readily ingested molluscicidal bait poison includes an inert mollusc bait and two active ingredient precursors. These precursors are edible and non-toxic to terrestrial molluscs when consumed alone. However, the composition which includes the two precursors is fatally toxic to terrestrial molluscs. One precursor is a simple iron compound, while the other precursor is selected from edetic acid, its hydroxyethyl derivatives and salts of these acids. In another embodiment a single active ingredient may replace the two precursors. This active ingredient may include ferric edetate and the ferric hydroxyethyl derivative of edetic acid.

Inventors: **Puritch; George S.** (Saanichton, **CA**); **Almond; David S.** (Victoria, **CA**); **Matson; Robert M.** (Victoria, **CA**); **Mason; Wenda M.** (Saanichton, **CA**)

Assignee: **W. Neudorff GmbH KG** (Emmerthal, **DE**)

Appl. No.: **295606**

Filed: **August 25, 1994**

Current U.S. Class: **424/408** ; 424/404; 424/410; 424/84

Field of Search: 424/404,84,410,408 514/492

References Cited [Referenced By]

U.S. Patent Documents

4765979	Aug., 1988	Nielsen
4983390	Jan., 1991	Levy
5290557	Mar., 1994	Mason et al.
5362749	Nov., 1994	Henderson et al.

Foreign Patent Documents

2207866A	Nov., 1994	GB

Other References

I F. Henderson, et al., Ann. Appl. Biol. (1990), 116, 273-278. .
I. F. Henderson, et al., Crop Protection, Apr. 1990, 9 131-134. .
I. F. Henderson, et al., 1989 BCP Mono. No. 41 Slugs and Snails in World Agriculture 289-294. .
Hendersen, et al., Aspects of Appl. Biol. (1986) 13, 341-347..

Primary Examiner: Page; Thurman K.
Assistant Examiner: Benston, Jr.; William E.
Attorney, Agent or Firm: Lahive & Cockfield Geary, III; William C.

Claims

What is claimed is:

1. A terrestrial mollusc stomach poison composition, comprising

a simple iron compound selected from the group consisting of iron proteins, iron carbohydrates, and iron salts, which, alone, have little or no toxicity to the molluscs;

a second component selected from the group consisting of edetic acid, hydroxyethyl derivative of edetic acid, or salts thereof; and

an inert carrier material edible to molluscs, wherein the molar ratio of the iron in the simple iron compound to the second component is in the range of 1:0.2 to 1:2.0.

2. The composition of claim 1 wherein the simple iron compound is present in an amount such that the iron concentration within the composition is in the range of about 200-10,000 ppm.

3. The composition of claim 1 wherein the second component is present at a concentration in the range of 2000 to 20,000 ppm

4. The composition of claim 1 wherein the simple iron compound is present in an amount such that the iron concentration within the composition is in the range of about 2000 to 6,000 ppm and the second component is present at a concentration of about 7,000 to 17,000 ppm.

5. The composition of claim 1 wherein the second component, in its salt form, is selected from the group consisting of calcium disodium edetate, monosodium edetate, disodium edetate, trisodium edetate, tetrasodium edetate, calcium disodium hydroxyethylethylenediaminetriacetate, monosodium hydroxyethylethylenediaminetriacetate and trisodium hydroxyethylethylenediaminetriacetate.

6. The composition of claim 1 wherein the simple iron compound is selected from the group consisting of saccharated ferric oxide, ferric albuminate, ferric ammonium citrate, ferric chloride, ferric citrate, ferrous gluconate, ferrous lactate, ferric phosphate, ferrous phosphate, ferric pyrophosphate, ferric nitrate, ferrous sulfate, iron stearate, and ferric tartrate.

7. The composition of claim 1 wherein the inert carrier is selected from the group consisting of wheat

cereal, agar, gelatin, oil cake, pet food wheat, soya, oats, corn, rice, fruits, fish by-products, sugars, coated vegetable and cereal seeds, casein, blood meal, bone meal, yeast, and fats.

8. A terrestrial mollusc stomach poison composition, comprising:

an effective amount of an active ingredient selected from the group consisting of ferric edetate and a ferric hydroxyethyl derivative of edetic acid.

9. The composition of claim 8 wherein the active ingredient is present in an amount such that the iron concentration is in the range of about 200-10,000 ppm.

10. The composition of claim 8 wherein the inert carrier is selected from the group consisting of wheat cereal, agar, gelatin, oil cake, pet food wheat, soya, oats, corn, rice, fruits, fish by-products, sugars, coated vegetable and cereal seeds, casein, blood meal, bone meal, yeast, and fats.

Description

BACKGROUND OF THE INVENTION

This invention relates to ingestible compositions for the control of terrestrial molluscs.

Terrestrial pulmonate gastropods, slugs and snails (collectively, molluscs) are significant plant pests that affect commercial agriculture and horticulture and domestic gardens. These molluscs are omnivorous and consume large amounts of vegetative materials during their daily foraging. Consequently, they can seriously damage vegetable gardens and even plant crops during all phases of the growing cycle. Because of their destructive potential, control measures must be employed to ensure adequate protection of the growing plants from damage by terrestrial molluscs.

A wide variety of approaches have been used to try to combat pest molluscs. Perhaps the most common is the use of poisonous compounds called molluscicides. Molluscicides include a diversity of chemical compounds such as table salt (NaCl), calcium arsenate, copper sulfate, and metaldehyde. Molluscicides fall into two major groups, depending upon their mode of action: contact poisons and ingestible (or bait) poisons.

Contact poisons are molluscicides that, to be effective, must come into physical contact with the exterior of the mollusc, either by external application or through the action of the mollusc traversing a molluscicidal composition placed on the ground. The contact molluscicide is picked up by the proteinaceous slime coat of the mollusc and it builds up in the body of the mollusc until a lethal proportion is reached. One of the major drawbacks of contact molluscicides is that they have little effect if the molluscs do not physically contact the active chemical agent. If the molluscs are hidden or migrate into an area after a contact molluscicide is spread, the molluscs are unaffected. For these reasons, contact-acting mollusc poisons generally are considered to be unreliable.

Heavy metals, including zinc, aluminum, copper and iron, are all toxic to molluscs and are examples of compounds known to be effective molluscicides when used as contact poisons in the form of salts or chelates. See, Henderson, et al. Crop Protection (1990), 9, 131-134 and Henderson, et al., Ann. Appl. Biol. (1990), 116, 273-278.

Ingestible (or bait) mollusc poisons are those that must be ingested by a mollusc in order to be lethal.

This type of mollusc poison tends to be preferred over contact poisons only because contact poisons, which rely upon passive acquisition of the active ingredient, are not considered to be reliable. One challenge associated with the development of effective bait molluscicides is to prepare a composition that is both palatable to the mollusc and effective as a lethal poison. Obviously, a sufficient quantity of the poison must be ingested to reach the lethal threshold. Often, compositions that are palatable to the mollusc are not effective as a lethal poison, while compositions that are quite potent and lethal are not readily ingested by molluscs. Many contact poisons, such as aluminum sulfate, copper sulfate and borax, are useless as ingestible poisons because they are not palatable to molluscs, and the molluscs do not ingest a lethal dose of these compounds. Ingestible poisons must be sufficiently palatable to the mollusc so that they will be consumed in lethal amounts, but the composition must also be slow acting enough to prevent the mollusc from becoming sick or cause it to cease feeding.

Typical problems associated with the development of compounds for the effective control of molluscs are discussed by Henderson, et al. in Aspects of Appl. Biol. (1986) 13, 341-347. This publication recognizes that although many compounds are known to be poisonous to molluscs, there is considerable difficulty in delivering the poison to the mollusc either as a bait or as a contact poison. The potential toxicity of a compound is irrelevant if molluscs will not consume a lethal dose of a bait poison.

One of the few compounds that act as both a contact and bait poison for terrestrial molluscs is metaldehyde. This compound is commonly used as a long lasting bait, attracting the molluscs and killing them after they ingest the poison bait. Despite its high effectiveness and its commercial popularity, metaldehyde is toxic to higher mammals and is a major contributor to domestic animal poisoning in the U.S. and Europe. More recently, U.K. Patent Application 2 207 866A has reported that specific complexes of aluminum with pentanedione compounds and iron with nitroso compounds would act as both ingested and contact poisons.

There is thus a need to develop an effective ingestible poison for molluscs that is palatable to molluscs and that does not pose a threat to the environment, crops, animals and other non-pests.

Accordingly, it is an object of the invention to provide a toxic, ingestible composition that is palatable to terrestrial molluscs. Another object is provide such a composition that poses no significant threat to the environment, crops, animals, or other non-pests. Other objects will be apparent upon review of the following description.

SUMMARY OF THE INVENTION

The invention provides an effective ingestible poison that is lethal to terrestrial molluscs. The composition is comprised of constituent compounds which do not pose any significant threat to the environment, plants, animals and other non-pests. In one embodiment the composition combines an inert carrier, such as a bait, with a simple iron compound and a second component. The simple iron compound can be an iron protein, an iron carbohydrate or an iron salt. The second component may be edetic acid, or hydroxyethyl derivative of edetic acid or a salt of these acids. Individually, neither the simple iron compound nor the second component is toxic to terrestrial molluscs. It is believed that the composition becomes toxic to molluscs only after it is ingested by the molluscs. Preferably, the molar ratio of iron in the simple iron compounds to the second component is in the range of 1:0.2 to 1:2.0. Preferably, the iron component is present in an amount such that the concentration of iron within the composition is in the range of about 200 to 10,000 ppm.

In another embodiment the composition comprises a single active ingredient in combination with an inert ingredient such as a mollusc bait. The single active ingredient may be ferric edetate or a ferric hydroxyethyl derivative of edetic acid. Preferably, the active ingredient is present in an amount such that

the concentration of iron within the composition is in the range of about 200 to 10,000 ppm.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a bait poison that is effective against terrestrial molluscs. In one embodiment the composition of the invention combines an inert, edible mollusc bait with two active ingredient precursors. Individually the active ingredient precursors are not toxic to the molluscs. It is only when the entire composition, including the active ingredient precursors, is ingested by molluscs that molluscicidal activity is achieved.

One active ingredient precursor is a simple iron compound. The simple iron compound can be an iron protein, an iron carbohydrate or an iron salt. A second active ingredient precursor is selected from the group consisting of edetic acid a hydroxyethyl derivative of edetic acid, or salts of these acids.

In another embodiment the molluscicidal composition combines an inert, edible mollusc bait with an active ingredient such as ferric edetate or a ferric hydroxyethyl derivative of edetic acid.

An advantage of the molluscicidal composition of the present invention is that it exhibits good mortality against terrestrial molluscs and it is readily consumed by terrestrial molluscs. A further advantage of this composition is that the constituents of the composition are environmentally safe and pose no threat to humans, animals or other non-pests. In fact, with the exception of the iron salts of edetic acid or hydroxyethyl derivatives of edetic acid the individual components are non-toxic to molluscs when administered alone. The composition of the invention not only is lethal to molluscs, but molluscs are also poisoned to the extent that they cease feeding upon plants after consuming the composition.

Preferably, the molar ratio of iron in the simple iron compound to the second precursor ingredient is in the range of 1:0.2 to 1:2.0.

The simple iron compound can be selected from any one of a number of iron salt compounds including iron proteins, iron carbohydrates, and iron salts. The iron compound can be present in its iron (II) state (ferrous) as well as in its iron (III) state (ferric). Examples of suitable simple iron compounds are saccharated ferric oxide, ferric albuminate, ferric ammonium citrate, ferric chloride, ferric citrate, ferrous gluconate, ferrous lactate, ferric phosphate, ferrous phosphate, ferric pyrophosphate, ferric nitrate, ferrous sulfate, ferric stearate, ferrous stearate, and ferric tartrate. One characteristic of the simple iron compounds used as an active ingredient precursor in this invention is that they have little or no toxicity to the molluscs when used alone. Suitable simple iron compounds are commercially available from a variety of sources, including Dr. Paul Lohmann GmbH KG of Emmerthal, Germany.

The simple iron compound preferably is present within the composition at an amount such that the iron concentration in the composition is in the range of about 200 to 10,000 ppm. More preferably, the simple iron compound should be present in an amount such that the iron concentration in the composition is in the range of 2000 to 6000 ppm.

As noted above, the second active ingredient precursor can be edetic acid, hydroxyethyl derivative of edetic acid or salts of these acids. Preferred salts of these acids include the sodium salts, such as calcium disodium edetate, monosodium edetate, disodium edetate, trisodium edetate, tetrasodium edetate, calcium disodium hydroxyethylethylenediaminetriacetate, monosodium hydroxyethylethylenediaminetriacetate, and trisodium hydroxyethylethylenediaminetriacetate. The second active ingredient precursor preferably is present in the composition at a concentration in the range of about 2000 to 20,000 ppm More preferably this component is present at about 7,000 to 17,000 ppm.

In the embodiment of the invention in which the bait molluscicide includes a single active ingredient such as ferric edetate or the ferric hydroxyethyl derivative of edetic acid, this active ingredient preferably is present in a level such that the iron concentration is in the range of about 200 to 10,000 ppm.

The single active ingredients are available from a variety of commercial sources. One commercial source for ferric sodium edetate is Dr. Paul Lohmann GmbH KG of Emmerthal, Germany which sells ferric edetate (Lohmann ferric edetate). In addition, ferric edetate is commercially available from the Hampshire Chemical Unit of W. R. Grace & Co. of Lexington, Mass. under the mark Hamp-Ene.RTM.. The hydroxyethyl derivative of ferric edetate is also available from the same unit of W. R. Grace under the marks Hamp-01.RTM. and Hampshire.RTM..

The inert bait component of the molluscicidal composition of the invention is one that must be readily consumed by molluscs. A variety of mollusc baits are well known and may be used in the compositions of the present invention. Such baits include agar, potato dextrose agar, gelatin, oil cake, pet food, wheat, soya, oats, corn, rice, fruits, fish by-products, sugars, coated vegetable and cereal seeds, casein, blood meal, bone meal, yeast, fats, and a variety of cereals, including wheat cereal. A preferred bait is wheat cereal which is commercially available from various sources.

The molluscicidal bait composition of the invention may also include additional formulation enhancing additives. Such additives include preservatives or anti-microbial agents, phagostimulants, water-proofing agents, and taste altering additives.

A variety of preservatives can be used effectively with this molluscicidal bait composition. Examples of preferred preservatives include Legend MK.RTM., available from Rohm & Haas Company of Philadelphia, Pa. and CA-24, available from Dr. Lehmann and Co. of Memmingen/Allgau, Germany. Preservatives such as these can normally be mixed with water to form a stock solution to be added to the formulation at a concentration in the range of about 10-750 ppm.

Phagostimulants can be added to the composition to attract molluscs and to induce molluscs to feed upon the composition. A variety of phagostimulants can be used, including sugars, yeast products and caesin. Sugars, such as sucrose, are among the more preferred phagostimulants. These additives are normally incorporated within the composition in a dry form. Typically, they can be added to the composition at about 1 to 2.5% by weight of the total composition.

Waterproofing agents, which can also act as binders, can be added to the composition to improve the weatherability of the molluscicidal bait. These are typically water insoluble compounds such as waxy materials and other hydrocarbons. Examples of suitable waterproofing agents are paraffin wax, stearate salts, beeswax, and similar compounds. One preferred wax compound is PAROWAX.RTM., available from Conros Corp. of Scarborough, Ontario, Canada. Waterproofing agents can be incorporated into the composition, in dry form, at about 5 to 12% by weight of the total composition.

It is also desirable to include within the molluscicidal bait taste altering compounds that render the composition unpalatable to animals. Exemplary compositions include those having a bitter taste. Suitable compounds that are commercially available include BITREX, available from Mcfarlane Smith Ltd. of Edinburgh, Scotland. These compounds typically are added at very low concentrations. For example, a 0.1% BITREX solution can typically be added to the composition at about 1 to 2% by weight of the total composition.

The molluscicidal bait of this invention typically is used in dry form and many of the constituent

ingredients of the composition are included in dry form. However, it is useful to include a sufficient amount of water within the composition to form a dough so that the ingredients can be more easily formed. Water is typically added at about 15 to 60% by weight of the total composition. The water, however, typically is driven off by heating and drying the molluscicidal bait before it is used.

As noted above, the compositions of the present invention are typically used in a dry, spreadable form such as powders, granules, cubes, or pellets. The composition may be spread on or around areas infested by molluscs as well as in areas in which mollusc infestation is to be prevented.

Dry molluscicidal compositions according to the present invention can be prepared as follows.

A suitable amount of the active ingredient precursors, or the active ingredient, is blended, in dry form, with a dry mollusc bait, such as wheat flour. Thereafter, other dry ingredients (such as phagostimulants and waterproofing agents) are blended and mixed with the bait. Next, suitable amounts of liquid additives (such as preservatives, taste altering additives and water) are added to the dry mixture to form a dough. The bait can be covered, such as with plastic wrap, and heated. One preferred heating technique is by heating in a microwave oven for 30 seconds to 10 minutes. After heating, the dough can be processed in a food grinder to obtain strands of the bait material. This is then dried, at elevated or ambient temperatures, and can be made into a desired form, such as powder, pellets or granules.

An exemplary formulation of a suitable mollusc bait is as follows.

The barrier composition of the present invention is effective against a variety of terrestrial molluscs including Ariolimax spp.; Arion species including, Arion ater, A. hortensis, A. rufas, A. circumcriptus, A. empericorum; Deroceras spp.; Agriolimax spp.; Prophysaon spp.; Helix pomata; and Cepaea nemoralis.

The following examples serve to further illustrate the invention.

EXAMPLE 1

Molluscicidal baits were prepared according to the general procedure discussed above. The active ingredient precursors were added in sufficient amounts to yield the concentrations noted in Table 1A. The iron based active ingredient precursor used was saccharated ferric oxide (iron sugar). The following additional ingredients were also included in the mixture: 2.5% by weight sucrose and 20 ppm Legend MK.RTM. antimicrobial agent, and 10% by weight paraffin wax. The control was prepared in a similar manner, except that it did not include the active ingredient precursors.

Tests were conducted in 25cm.times.50cm.times.5cm planting trays (two trays per treatment with 5 slugs per tray). Each tray was floored with wet potting soil and covered with a transparent, plastic lid. Each tray received five garden slugs, Arion ater. Ten grams of each of the formulations identified below in Table 1A were placed inside a petri dish and put in each planting tray along with a lettuce plant. The planting trays were placed outside in the shade during the course of the experiment. Table 1B illustrates the observed mortality (slugs killed/5) and percent of bait eaten for each formulation, at 6 days after testing (DAT).

EXAMPLE 2

The formulations identified below in Table 2A were prepared by the general procedure noted above. The active ingredient precursors were used in sufficient amounts to yield the concentrations noted in Table 2A. The iron-based active ingredient precursor used was saccharated ferric oxide. Additional ingredients

used in the formulations included 10% by weight paraffin wax, 2.5% by weight sucrose and 20 ppm Legend MK.RTM..

Test were conducted in 36 liter plastic tubs (two tubs per treatment with ten slugs per tub). Each tub was floored with wet potting soil and covered with a lid. Each tub received ten garden slugs, Arion ater. Ten grams of each molluscicidal bait identified in Table 2A was added to two petri dishes and placed in a tub along with two lettuce plants. The tubs were left outdoors during the course of the experiment.

The tubs were examined three days after treatment and seven days after treatment to assess the slug mortality (slugs killed/10) and amount of each molluscicidal bait eaten. These data are presented in tables 2B and 2C. Bait consumption is rated according to the following standards: heavy, greater than 20% bait consumed; moderate, 10 to 20% bait consumed; and light, less than 10% bait consumed.

EXAMPLE 3

Compounds identified below in Table 3A were prepared according to the general procedure noted above. The active ingredient precursors were added in sufficient quantities to yield the concentration noted in Table 3A. The following additional ingredients were also added for each formulation: 10% by weight of paraffin wax, 2.5% by weight sucrose and 20 ppm Legend MK.RTM..

Tests were conducted in 25 cm.times.50cm.times.5cm planting trays (two trays per treatment with 5 slugs per tray). Each tray was floored by wet potting soil and covered by transparent plastic lids. Each tray received 5 garden slugs, Arion ater, and ten grams of bait which was placed inside a petri dish and placed on the soil along with a lettuce plant. The trays were placed outside in the shade during the course of the experiment.

The slug mortality (slugs killed/5) and percent of bait consumed was evaluated at 6 DAT and these data are shown in Table 3B.

EXAMPLE 4

The formulations identified in Table 4A were prepared according to the general procedure noted above. The active ingredient precursors were added in sufficient amounts to yield the concentration shown in Table 4A. Additional ingredients for each formulation included 10% by weight paraffin wax, 2.5% by weight sucrose, and 20 ppm Legend MK.RTM..

Tests were conducted in 36 liter plastic tubs (2 tubs per treatment with 10 slugs per tub). Each tub was floored by wet potting soil and covered with a plastic lid. Each tub received 10 garden slugs, Arion ater, and ten grams of bait, which was placed inside a petri dish. The petri dishes were placed on the soil along with two lettuce plants. The tubs were left outside during the course of the experiment. The tubs were examined at 1 DAT and 6 DAT to determine slug mortality (slugs killed/10) and the percent of the bait consumed. These data are illustrated below in Tables 4B and 4C.

EXAMPLE 5

The bait compositions identified in Table 5A were prepared according to the general procedure noted above. Active ingredient precursors were added in sufficient amounts to yield the concentrations noted. Additional ingredients included 10% paraffin wax, 2.5% by weight sucrose and 20 ppm Legend MK.RTM..

The tests were conducted using 36 liter plastic tubs (2 tubs per treatment with 10 slugs per tub). Each

tub was floored by wet potting soil and covered with a plastic lid. Each tub received 10 garden slugs, Arion ater, and ten grams of bait which was placed inside a petri dish and placed on the soil in each tub along with two lettuce plants. The tubs were left outside during the course of the experiment.

The tubs were examined at 1 DAT and 6 DAT to determine the slug mortality (slugs killed/10) and the amount of bait consumed. These data are illustrated in Tables 5B and 5C.

EXAMPLE 6

The bait compositions identified in Table 6A were prepared according to the general procedure noted above. Active ingredient precursors were added in sufficient amounts to yield the concentrations noted. Additional ingredients included 10% by weight paraffin wax; 2.5% by weight sucrose; and 20 ppm Legend MK.RTM..

Feeding tests were conducted inside 36 liter plastic containers. Two containers were used for each treatment so that there were two replicates. Each container was filled to 3 cm with potting soil that was made damp. Ten slugs, Arion ater, were placed into each tub at the start of the experiment. At the same time, 10 grams of bait was placed into a petri dish and the dish was placed on the soil in the container. Two lettuce plants or one potted marigold plant were placed on their side and positioned on the soil within the container as an alternate food source. The containers were then covered with plastic lids and the tubs were placed outside in a shaded area. The containers were evaluated at 5 DAT and 6 DAT by counting all living and dead slugs and removing the dead slugs. At the same time, the plants and the bait were examined to determine the amount of bait consumed. Table 6B, below, illustrates the slug mortality and the bait consumption for replicates 1 and 2 at 5 DAT. Table 6C illustrates the total slug mortality for the two replicates.

EXAMPLE 7

The formulations shown in Table 7A were prepared according to the general procedure noted above. The tests were conducted using 1/2 liter food containers (2 containers per treatment with 3 slugs per container). Each container was floored by wet potting soil and covered with transparent, plastic lids. Each container received 3 garden slugs, Deroceras reticulatum, and a cube of bait placed directly on the soil. The containers were examined at 1 DAT and 5 DAT to determine the slug mortality and the bait consumption. Bait consumption was recorded according to a scale in which "heavy" indicates greater than 20 percent bait consumption; "moderate" indicates 10 to 20 percent consumption; and "light" indicates less than 10 percent bait consumption. The data obtained are illustrated in Tables 7B and 7C below.

EXAMPLE 8

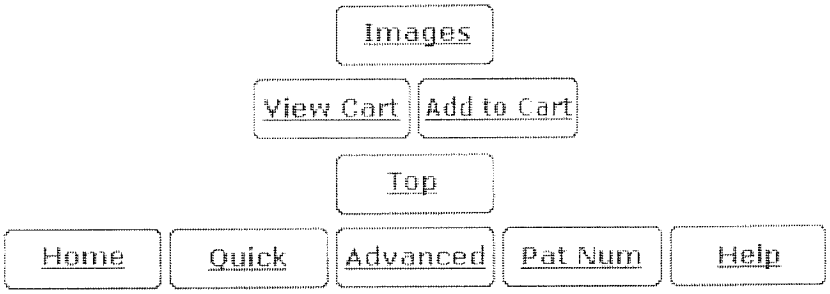
The baits identified in Table 8A were prepared according to the general procedure noted above. Hamp-Ene.RTM. ferric sodium edetate was obtained from W. R. Grace and Company of Lexington, Mass. and Lohmann ferric sodium edetate was obtained from Dr. Paul Lohmann GmbH KG of Emmerthal, Germany. Tests were conducted in 36 liter tubs (2 tubs per treatment with 10 Deroceras reticulatum per tub). Each tub had a soil covering the base of the tub and the tub was covered with a plastic lid. Three lettuce plants were placed in the tubs as an alternate feeding source. Slugs were introduced to the tubs at the time the baits were added.

The tubs were examined at 4 DAT to determine slug mortality. These data are shown in Table 8B.

One of ordinary skill in the art will appreciate that minor modifications may be made to the

compositions of the present invention without departing from its intended scope.

* * * * *



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AQUEOUS PHOTOLYSIS OF THE IRON (III) COMPLEXES OF NTA, EDTA AND DTPA.

Anders Svenson, Lennart Kaj and Håkan Björndal

SWEDISH ENVIRONMENTAL RESEARCH INSTITUTE

PO Box 21060
S-100 31 Stockholm
SWEDEN

Abstract

The rate of photolysis of iron complexes with three common complexing agents, NTA, EDTA and DTPA was analysed. Aqueous solutions of each complex were illuminated in a Xenotest 1200. Using a sun spectrum from 60°N (Stockholm latitude), environmentally related half lifetimes were calculated.

The half lifetimes were 43, 11 and 8 min for the three 1:1 complexes, resp., dissolved in the top layer of a water system and illuminated at the yearly maximum of sun light in the specified area. In the environment several factors operate to reduce this rate. However, photolytic degradation pathways seem to be an important environmental fate of these substances.

Introduction

Nitrilo-triacetic acid (NTA), ethylenediaminine-tetraacetic acid (EDTA), and diethylenetriamine-pentaacetic acid (DTPA) form strong complexes with several divalent and trivalent metal cations. The log K of their ferric complexes has been reported as 15.9, 25.1, and 28.6, respectively (1).

The complex formation has been used industrially in various applications. NTA and EDTA are important constituents of some detergents and DTPA is used in pulp and bleaching processes. When discharged and treated in municipal sewage plants only NTA will be bioconverted to any measurable degree, whereas EDTA and DTPA pass through the process undegraded (2,3). Under specific conditions the two latter substances, however, have been shown to be degraded by microorganisms (4).

Receiving waters normally contain ferric ions and considering the concentrations and association constants of metal ions in water, almost all emitted complexing agents will be bound to ferric ions. Ferric complexes absorb sunlight and the organic moiety will decompose. Thus EDTA has been shown to be degraded and form several conversion products (Fig. 1)(5). Splitting of acetic acid residues was an important conversion step.

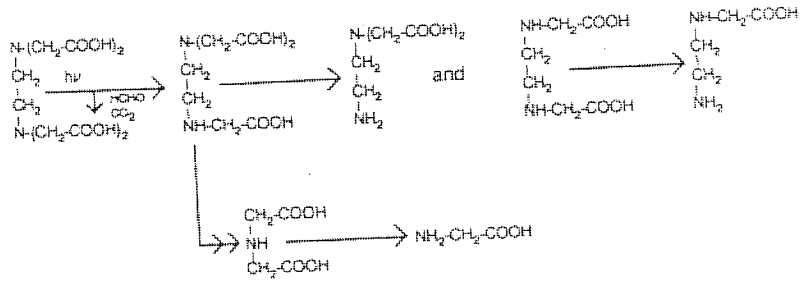

Fig. 1. Reaction scheme of the photochemical conversion of EDTA at neutral pH in the aquatic enviroment.

We present a kinetic study of the photochemical conversion of the ferric complexes of NTA, EDTA, and DTPA. With these substances we would like to emphasize the photolytic component of conversion of substances in the environment that together with biodegradation and chemical conversion reduce the persistence of a compound in the environment.

Materials and methods

Nitrilo-triacetic acid (NTA), ethylenediamine-tetraacetic acid, disodium salt (EDTA), and diethylenetriamine-pentaacetic acid (DTPA) were of p.a. quality and obtained from E. Merck AG, Darmstadt, FRG. Solutions of ferric 1:1 complexes (0.10 mM in 25 mM sodium phosphate, pH 7.00) were illuminated in a Xenotest 1200 apparatus, Original Hanau, in 1 x 1 x 4 cm^3 quartz cuvettes as described earlier (6,7).

Absorption spectra of the complexes were recorded in a Beckman DU-8 spectrofotometer. The incident light intensity was measured using a chemical actinometer composed of 10 mM uranyl nitrate in 50 mM oxalic acid (6-8). The oxalic acid concentration was analyzed in a total organic carbon-analyzer, Astro Model 1850 (7).

Concentrations of NTA, EDTA and DTPA were analyzed by gas chromatography as methyl esters essentially according to a published procedure (9). A 1 ml sample was added 25 µg cyclohexane-diamine tetraacetic acid as an internal standard and the solvent was evaporated to dryness using gaseous nitrogen. One ml 10 % borotrifluoride in methanol was added and the samples were treated one hour at 100°C. After cooling 1 ml of trichloromethane and 3 ml 1 M potassium phosphate, pH 7,0, was added, followed by extraction and separation of the two phases. The volume of the organic phase was reduced to about one third and from this solution samples were injected into the gas chromatograph (Hewlett-Packard 5880 A). The chromatograph was fitted with a splitless injector (injector temperature 250°C) and a 7.5 m x 0.31 mm SE-54 capillary column. A temperature program from 100°C to 290°C was run at 15°/min and the substances were detected by a flame ionisation detector (300°C).

Quantum yields of conversion and half lifetimes were calculated for the iron complexes of NTA, EDTA and DTPA as described earlier (6,7,10). A calculated sun spectrum representing daily and yearly maxima at 60°N (Stockholm latitude) was used as the environmental light source (7,11).

Results and discussion

Gas chromatographic analyses showed that NTA, EDTA and DTPA as their 1:1 ferric complexes were degraded in a light dependant process upon illumination a few minutes in a Xenotest 1200 apparatus. In Fig. 2 the rate of photolytic conversion is shown.

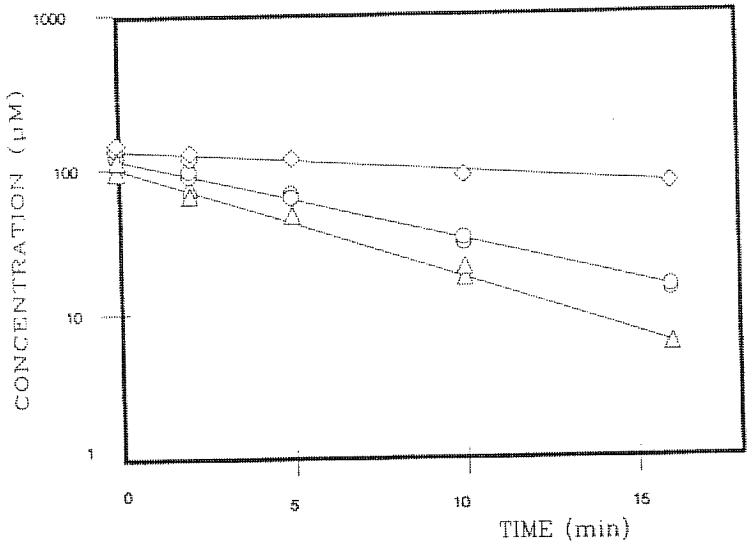

Fig. 2. Photochemical conversion of NTA (◇), EDTA (○), and DTPA (△) at pH 7,0 upon illumination in a Xenotest 1200.

From this rate and the rate of light absorption, quantum yields of conversion were calculated (Table 1). The results show that different proportions of the absorbed light energy were used to degradation of these complexing agents.

The calculated half lifetimes, as shown in Table 1, refer to the top millimeters of a waterbody in the Stockholm area at noon, June 21st. These estimates represent optimised conditions in various aspects and the true environmental lifetime certainly is longer. Such environmental photolytic lifetimes may be estimated taking a number of system-related factors into consideration (6,12,13). The following most important factors have to be considered: the daily and yearly periodicity of incident light, weather conditions like cloudiness, shadowing effects of surface and shore vegetation, absorption and scattering by suspended solids, dissolved organic compounds and planctic organisms.

Table 1. Photolytic conversion of the ferric 1:1 complexes of NTA, EDTA, and DTPA.

Complex	Conversion rate (molecules/ $cm^3 \times s$)	Light absorption (photons/ $cm^3 \times s$)	Quantum yield of conversion	Theoretical half lifetime* (min)
Fe - NTA	3.95×10^{13}	3.07×10^{15}	0.0129	42.9
Fe - EDTA	1.20×10^{14}	5.28×10^{15}	0.0243	11.3
Fe - DTPA	1.35×10^{14}	1.70×10^{16}	0.00793	8.04

* Calculated from the yearly maximum of a solar spectrum at 60°N.

Evaluation of the overall environmental lifetime of a substance usually takes only biodegradation into consideration. In activated sludge processes both EDTA and DTPA are not degraded, while NTA will decompose. A half lifetime for NTA degradation in such a process of 1.6 - 2.9 hours were calculated from data

presented elsewhere (2). As in the case of photolysis these biodegradation tests represent optimised conditions that hardly occur in the whole body of a receiving water.

The complexing agents NTA, EDTA and DTPA, as their ferric complex are photolytically degraded in the environment. The rates at opimised conditions in the top layers of a water body at the yearly maximun of sunlight are rapid with halflives shorter than 1 hour.

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PLANT PHYSIOLOGY

LINCOLN TAIZ
UNIVERSITY OF CALIFORNIA, SANTA CRUZ

EDUARDO ZEIGER
UNIVERSITY OF CALIFORNIA, LOS ANGELES

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carried out with impure salts that contained as contaminants other elements now known to be essential (such as boron or molybdenum). An example of a more modern formulation for a nutrient solution is shown in Table 5.4. This formulation is called a modified **Hoagland's solution**, named after Dennis R. Hoagland, a researcher prominent in the development of modern mineral nutrition research in the United States. This solution has all the elements know to be needed for optimal plant growth. An important property of this formulation is that its nitrogen is supplied as both ammonium and nitrate. Supplying nitrogen in a balanced mixture of cations and anions tends to reduce the rapid rises in the pH of the medium that are commonly observed when the nitrogen is supplied solely as nitrate anion (Asher and Edwards, 1983).

A significant problem in growing plants in nutrient solutions is that of supplying sufficient quantities of iron. When iron is supplied as an inorganic salt, such as $FeSO_4$ or $Fe(NO_3)_2$, it can precipitate out of solution as iron hydroxide. If phosphate salts are present, insoluble iron phosphate will also form. This precipitation of the iron out of solution makes it unavailable for the plant, unless iron salts are added at frequent intervals. Initial attempts to alleviate this problem involved adding iron together with citric acid or tartaric acid to the nutrient solution. These compounds, which form a soluble complex with cations such as iron and calcium, are called **chelating agents**. More modern formulations have used the chemical ethylenediaminetetraacetic acid (EDTA). The structure of EDTA is shown in Figure 5.10.

TABLE 5.4. Composition of a modified Hoagland's nutrient solution for growing plants*

Compounds added to nutrient solution	*Nutrient elements*	*Final concentration*	
		μm	*ppm*
Macronutrients			
KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, $NH_4H_2PO_4$, $MgSO_4 \cdot 7H_2O$	N	16,000	224
	K	6,000	235
	Ca	4,000	160
	P	2,000	62
	S	1,000	32
	Mg	1,000	24
Micronutrients			
KCl, H_3BO_4, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, H_2MoO_4, Fe-EDTA	Cl	50	1.77
	B	25	0.27
	Mn	2.0	0.11
	Zn	2.0	0.131
	Cu	0.5	0.032
	Mo	0.5	0.05
	Fe	20	1.12

Adapted from Epstein, 1972.
*The compounds are added separately from stock solutions to prevent nutrient precipitation during the preparation of the nutrient solution (see Epstein, 1972, for details).

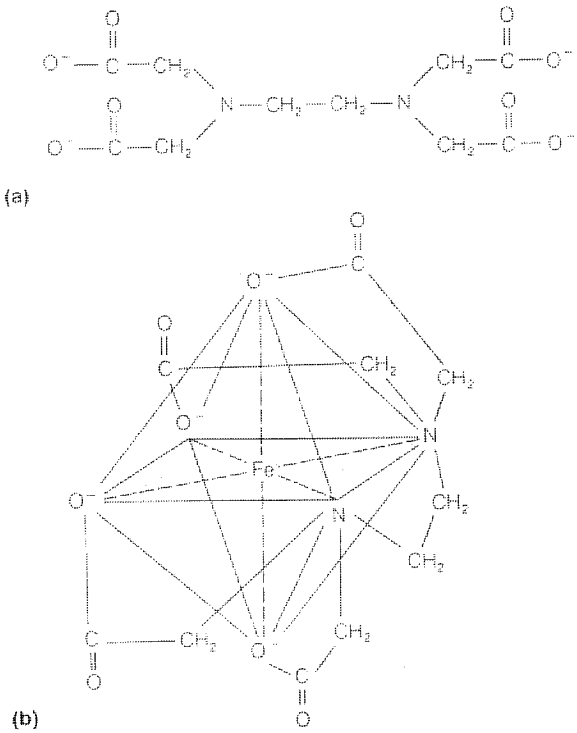


FIGURE 5.10. Chemical structure of the chelator ethylenediaminetetraacetic acid (EDTA) by itself (a) and chelated to an Fe^{3+} ion (b). Iron binds to EDTA through interaction with the two nitrogen atoms and the four ionized oxygen atoms of the carboxylate groups. The resulting ring structure clamps the metallic ion and effectively neutralizes its reactivity in solution. During the uptake of iron at the root surface, Fe^{3+} is reduced to Fe^{2+}, which is released from the EDTA-iron complex. The chelator can then bind other available Fe^{3+} ions.

Chelating agents contain negatively charged carboxyl groups or electron-donating nitrogen groups that can bind the iron in a coordination complex. Iron-EDTA complexes are commonly used in nutrient solutions for plants grown hydroponically, aeroponically, or in the soil. The fate of the chelation complex during iron uptake by the root cells is not clear; it has been suggested that during the absorption of iron by the roots, the iron is released from the chelator when it is reduced from Fe^{3+} to Fe^{2+} at the root surface. The chelator can then diffuse back into the nutrient (or soil) solution and react with another Fe^{3+} ion. Following uptake into root cells, iron is kept soluble by chelation with organic compounds present in plant cells. It appears that citric acid may play a major role in the chelation of iron and its long-distance transport in the xylem (Olsen et al., 1981).

Roles of Essential Elements and Nutrient Disorders

Inadequate supply of an essential element results in a nutritional disorder manifested by characteristic deficiency symptoms. In hydroponic culture a given symp-

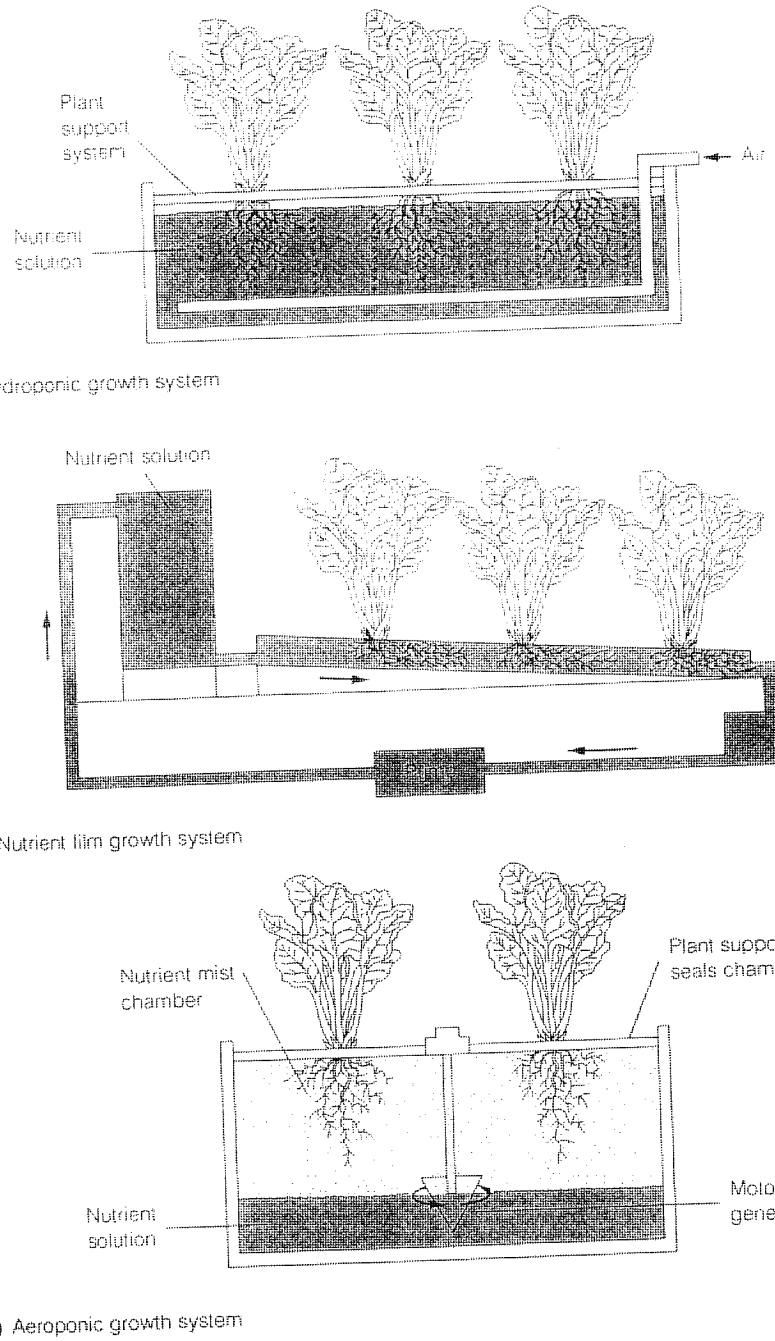


FIGURE 5.9. Hydroponic and aeroponic systems for growing plants in nutrient solutions in which composition and pH can be automatically controlled. In a hydroponic system (a), the roots are immersed in the nutrient solution, and air is bubbled through the solution. An alternative hydroponic system (b) often used in commercial production is the nutrient film growth system, in which the nutrient solution is pumped as a thin film down a shallow trough surrounding the plant roots. In this system, the composition and pH of the nutrient solution can be automatically controlled. In the aeroponic system (c), the roots are suspended over the nutrient solution, which is whipped into a mist by a driver attached to a motor shaft. (Aeroponic system adapted from Zobel et al., 1976.)

result of nutrient uptake by the roots. Hydroponics can be used for the commercial growth of plants such as lettuce. In commercial hydroponic culture, nutrient solutions are often recirculated in a thin layer that flows through a trough surrounding the plant roots (Asher and Edwards, 1983). With this method, called the "nutrient film technique" (Fig. 5.9b), nutrient solution composition and pH can be monitored continually and adjusted automatically.

One alternative to growing plants with their roots immersed in a nutrient solution is to grow them in a support material such as sand, gravel, or vermiculite. Nutrient solutions can then be added and old solutions can be removed by leaching. Another alternative is to grow the plants **aeroponically** (Zobel et al., 1976). In this technique, plants are grown with their roots suspended in air while being sprayed continuously with a nutrient solution (Fig. 5.9c). This approach ensures that the roots receive an ample supply of oxygen and avoids limitations on gas exchange.

Plant Physiologists Have Formulated Nutrient Solutions That Can Sustain Optimal Plant Growth

Over the years, many formulations have been used for nutrient solutions. Early formulations developed by Knop included only KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$, and an iron salt. At the time, this nutrient solution was believed to contain all the components required by the plant, but we know now that these experiments were

acetyl coenzyme A and *S*-adenosylmethionine. The latter compound is important in synthetic reactions involving the transfer of methyl groups, as in lignin synthesis (see Chapter 13).

Phosphate Assimilation

Phosphate in the soil solution is readily taken up by plant roots and incorporated into a variety of organic compounds including sugar phosphates, phospholipids, and nucleotides. The main entry point of phosphate into assimilatory pathways occurs during the formation of ATP, the energy "currency" of the cell. The overall reaction for this process involves the addition of inorganic phosphate to the second phosphate group in adenosine diphosphate to form a phosphate ester bond. In mitochondria, the energy for ATP synthesis is derived from the oxidation of NADH produced during the breakdown of sugars in respiration, a process called oxidative phosphorylation (Chapter 11). The formation of ATP is also driven by light energy in the process of **photophosphorylation** that occurs in the chloroplasts (Chapter 8). In addition to these reactions in mitochondria and chloroplasts, phosphate is assimilated by reactions in the cytosol. In glycolysis, inorganic phosphate is incorporated into 1,3-bisphosphoglyceric acid, forming a high-energy acyl phosphate group. This phosphate can be donated to ADP to form ATP in a **substrate-level phosphorylation** reaction (Fig. 12.15). Once incorporated in ATP, the phosphate group can be transferred in reactions to form the various phosphorylated compounds found in higher plant cells.

Cation Assimilation

Assimilation of cations taken up by plant cells involves the formation of complexes with organic compounds without any chemical transformation of the nutrient. That is, the cation becomes bound to the carbon compound without the formation of covalent bonds. This occurs for macronutrient cations such as potassium, magnesium, and calcium as well as for micronutrient cations such as copper, iron, manganese, cobalt, sodium, and zinc. The assimilation of iron will be discussed in more detail in the next section.

Cation Complexes with Carbon Compounds Involve the Formation of Coordination or Electrostatic Bonds

In the formation of a coordination complex, oxygen or nitrogen atoms of a carbon compound donate unshared electrons to form a bond with the cation nutrient. As a result, the positive charge on the cation is neutralized. Coordination complexes typically occur between polyvalent cations and organic molecules, such as copper and tartaric acid (Fig. 12.16a) and magnesium and chlorophyll *a*, in which the magnesium is bound by coordination bonds with the nitrogen group atoms in the porphyrin ring (Fig. 12.16b). The nutrients that are assimilated with the formation of coordinate complexes include copper, zinc, iron, and magnesium. Calcium can also form coordinate complexes with cell wall polygalacturonic acid (Fig. 12.16c).

Electrostatic bonds are formed because of the attraction of a positively charged cation for a negatively charged group on an organic compound. In electrostatic bonds, unlike coordinate bonds, the cation retains its positive charge. An important negatively charged group is the ionized form of a carboxylic acid. For example, monovalent cations such as potassium can form electrostatic bonds with the carboxylic groups of many organic acids, as illustrated in Figure 12.17a. The incorporation of calcium into the pectic components of the cell wall involves coordinate bonds as well as electrostatic bonds with the carboxylic groups of polygalacturonic acid (Fig. 12.17b). In general, assimilation of magnesium and calcium that is not accounted for by the formation of coordination complexes takes place through the forma-

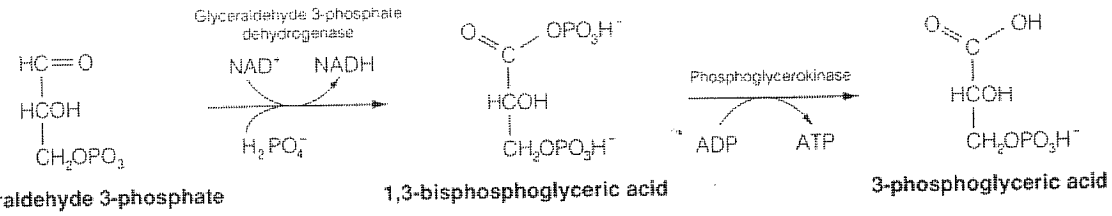
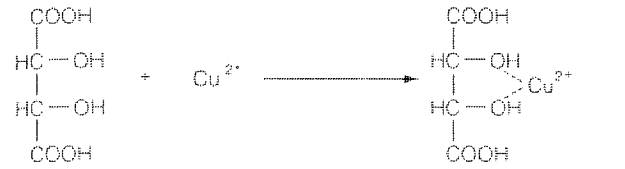


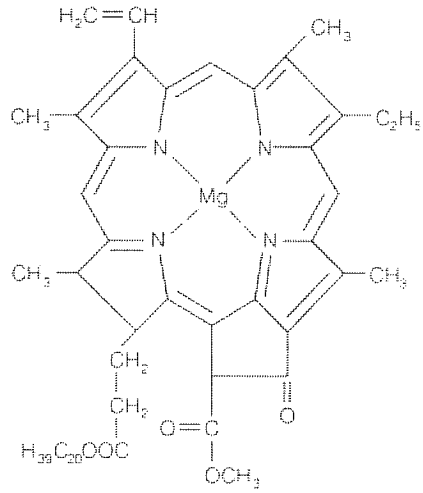
FIGURE 12.15. Substrate-level phosphorylation with the production of ATP. This reaction, which occurs in glycolysis, involves the incorporation of inorganic phosphate into 1,3-bisphosphoglyceric acid (first reaction) and the breakdown of this compound to produce ATP from ADP.

FIGURE 12.16. Examples of coordination complexes. (a) Copper ions share electrons with the hydroxyl oxygens of tartaric acid. (b) Magnesium ions share electrons with nitrogen atoms in chlorophyll a. (c) The "eggbox" model of the interaction of polygalacturonic acid and calcium ions. On the left, calcium ions are held in the spaces between two polygalacturonic acid chains, indicated by the kinked, horizontal lines. The arrow shows a blow-up of a single calcium ion forming a coordination complex with the hydroxyl oxygens of the galacturonic acid residues. Much of the calcium in the cell wall is thought to be bound in this fashion (From Rees, 1977.)

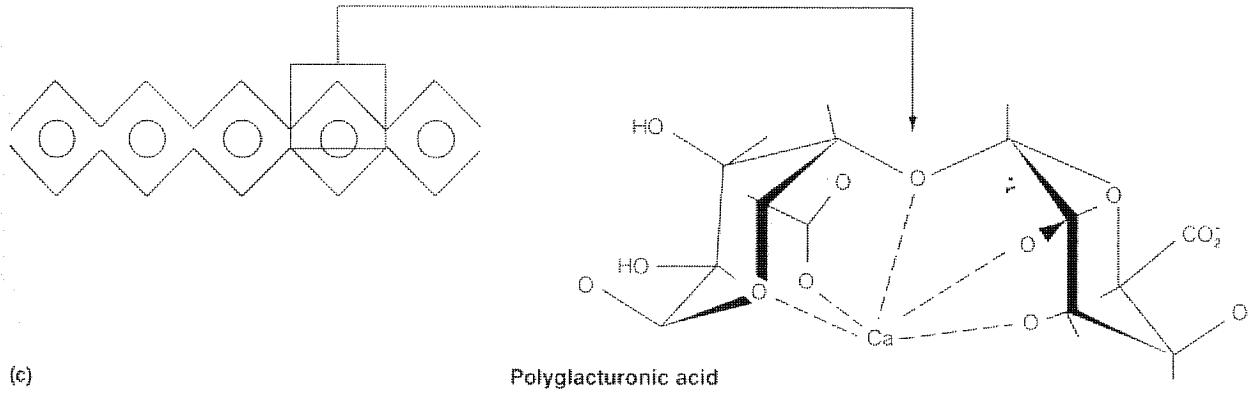


Tartaric acid — Copper-tartaric acid complex

(a)



(b) Chlorophyll *a*



(c) Polygalacturonic acid

tion of electrostatic bonds with amino acids, phospholipids, and other negatively charged molecules. It should be noted that much of the potassium that is accumulated by plant cells remains as the free ion in the cytosol and the vacuole and functions in osmotic regulation and enzyme activation.

Iron Assimilation Involves Redox Reactions and Complex Formation

Iron is important in plant cells as a catalyst in enzyme-mediated redox reactions and in chlorophyll biosynthesis (Bienfait and Van der Mark, 1983). Iron is acquired by plant roots from the soil, where it is present primarily in the ferric form (Fe^{3+}) as oxides such as $Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$ (Olsen et al., 1981). This form of iron is extremely insoluble, as indicated by the K_{sp} (2×10^{-39}), which describes the equilibrium between the solid and dissolved species. It is therefore difficult for plant roots to obtain iron from the soil. The process by which plant roots can solubilize and acquire iron has been studied in detail for dicots. In response to iron stress, these plants: (1) acidify the medium surrounding the root, (2) chemically reduce soluble ferric chelates (Fe coordination complexes), (3) take up ferrous iron (Fe^{2+}), and (4) accumulate organic acids such as citrate (Bienfait and Van der Mark, 1983). Acidification and reduction are key events in the solubilization of iron, since acidification of the soil surrounding the roots greatly increases the solubility of the ferric form of iron (Olsen et al., 1981) and the reduced ferrous iron (Fe^{2+}) is much more soluble than the oxidized ferric form (Fe^{3+}). There has been a controversy as to whether iron reduction takes place as a result of the release of "reducing substances" such as caffeic acid into the soil from root cells (Olsen et al., 1981) or whether reduction occurs on the membrane surface of root cells by the

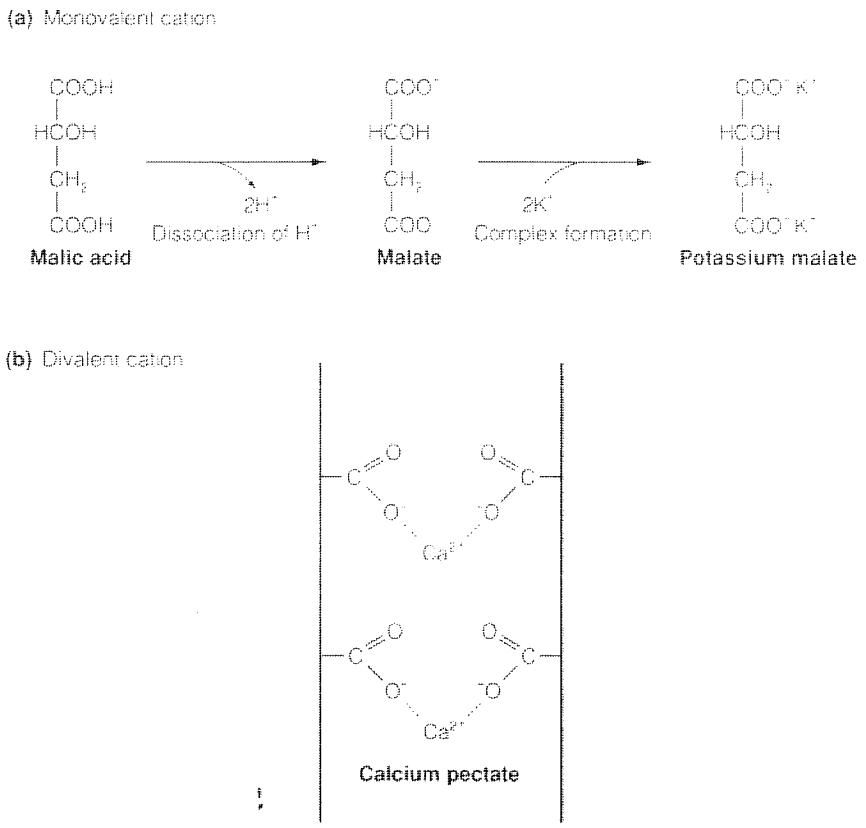


FIGURE 12.17. Examples of the formation of electrostatic (ionic) complexes. (a) Monovalent cation, potassium malate. (b) Divalent cation, calcium pectate. Divalent cations can form cross-links between parallel strands containing negatively charged carboxyl groups. Calcium cross-links may play a structural role in the cell walls of some algae and higher plants.

action of a redox electron transport chain (Bienfait and Van der Mark, 1983). Under conditions of iron deficiency, the activity of a trans-plasma membrane electron transport system greatly increases in bean roots and certain other species and may represent an adaptation to low Fe^{3+} concentrations in the soil (Crane et al., 1985). In any case, reduction appears to be a prerequisite for the uptake of iron into plant cells (Chaney and Brown, 1972). Most evidence suggests that the iron is then reoxidized in the cells of the root and transported to the leaves as an electrostatic complex with citrate (Olsen et al., 1981).

When iron-deficient plants receive iron, they may often take up massive amounts by an augmentation of the uptake system. Large amounts of "free" iron in plant cells can pose problems because iron can interact with oxygen to form superoxide anions (O_2^-), which can damage membranes by degrading unsaturated lipid components (Halliwell, 1974; Trelstad et al., 1981). Plant cells can, however, provide safe storage for iron in the leaves through the use of an iron storage protein called **phytoferritin** (Bienfait and Van der Mark, 1983). Phytoferritin is made up of 24 identical protein subunits that form a hollow sphere with a molecular mass of about 480,000 Da. Iron incorporation into phytoferritin is thought to occur by oxidation of ferrous iron inside the phytoferritin protein and formation of an iron core (Bienfait and Van der Mark, 1983). The core consists of about 5400 to 6200 iron atoms present as a ferric oxide-phosphate complex. How iron is released from phytoferritin is uncertain, but it is generally thought to involve a breakdown of the protein complex. It is also thought that the level of free iron in plant cells may regulate the de novo biosynthesis of phytoferritin (Zähringer et al., 1976).

When iron acts as a redox component, it is often present as a complex within a porphyrin group in the active site of an enzyme. This is the case, for example, in the cytochromes that mediate electron transport in the mitochondrial inner membrane. Therefore, an important assimilatory reaction for iron involves its insertion into the porphyrin system. This reaction is called the ferrochelase reaction, and it is catalyzed by the enzyme ferrochelase (Fig. 12.18) (Jones, 1983). Ferrochelase is associated with chloroplasts and mitochondria and most

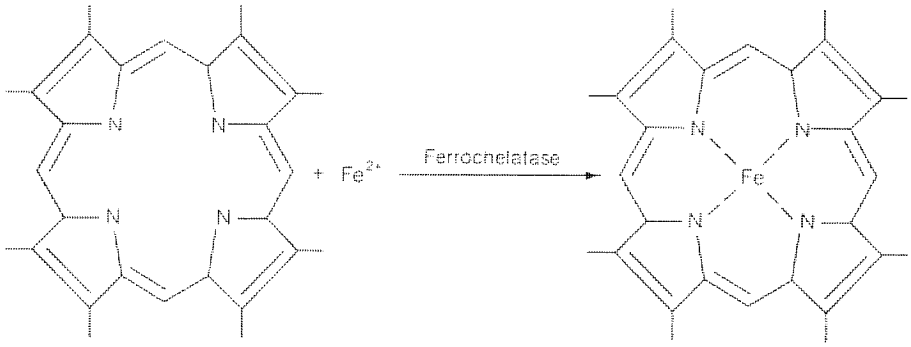


FIGURE 12.18. The ferrochelase reaction. The enzyme ferrochelase catalyzes the insertion of iron into the porphyrin ring to form a coordination complex.

likely functions in active metalloporphyrin synthesis for electron transport systems in these organelles. In addition, iron-sulfur proteins of the electron transport chain (Chapter 9) contain Fe covalently attached to the cysteine sulfur atoms of the apoprotein, as well as Fe atoms bound to other Fe atoms by means of sulfur bridges.

Oxygen Assimilation

Although respiration accounts for the bulk of the oxygen (O_2) utilized by plant cells, a small proportion of oxygen may be directly assimilated into organic compounds in the process of **oxygen fixation**. Oxygen fixation involves direct addition of molecular oxygen to an organic compound in reactions carried out by enzymes known as oxygenases (Metzler, 1977). Other than oxygen fixation, the major pathway for the assimilation of oxygen into organic compounds involves reactions in which the oxygen from water is incorporated. For many years, it was assumed that only the latter pathway occurs in living organisms.

Oxygenases are classified as **dioxygenases** or **monooxygenases** according to the number of atoms of oxygen that are transferred to an organic compound in the enzyme-catalyzed reaction. Examples of dioxygenases in plant cells are lipoxygenase, which catalyzes the addition of two atoms of oxygen to unsaturated fatty acids, and prolyl hydroxylase, the enzyme that converts proline to the rare amino acid hydroxyproline (Fig. 12.19). Hydroxyproline is an important component of cell wall protein, **extensin** (see Chapter 1). Prolyl hydroxylase is considered a dioxygenase because one oxygen atom is incorporated into proline to form hydroxyproline, while a second oxygen is used to convert α-ketoglutarate to succinate. Ferrous iron and ascorbate are also needed as cofactors but do not participate directly in the redox reaction (Fig. 12.19b). The synthesis of hydroxyproline from proline differs from the synthesis of all other amino acids in that the reaction occurs *after* the proline has been incorporated into protein and is therefore a post-translational modification reaction. Prolyl hydroxylase is localized in the endoplasmic reticulum, suggesting that most, if not all, proteins containing hydroxyproline are found in the secretory pathway.

Monooxygenases add one of the atoms in molecular oxygen to an organic compound; the other oxygen atom is converted into water. Monooxygenases are sometimes referred to as *mixed-function oxidases* because of their ability to catalyze simultaneously both the oxygenation reaction and the oxidase reaction (reduction of oxygen to water). The monooxygenase reaction also requires a reduced substrate (NADH or NADPH) as an electron donor, according to the following equation:

$$A + O_2 + BH_2 \rightarrow AO + H_2O + B$$

where A represents an organic compound and B represents the electron donor. An important monooxygenase in plant cells is the family of heme proteins collectively called cytochrome P-450, which catalyzes the hydroxylation of cinnamic acid to *p*-coumaric acid. In monooxygenases, the oxygen is first activated by combining with the iron atom of the heme group. NADPH serves as the electron donor, as shown in Figure 12-19c. The mixed-function oxidase system is localized on the endoplasmic reticulum and is capable of oxidizing a variety of substrates, including mono- and diterpenes and fatty acids.

Another reaction in which oxygen is directly incorporated into an organic compound occurs during photorespiration (see Chapter 9) and involves the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the enzyme of CO_2 fixation (Ogren, 1984). The first stable product that contains oxygen originating from molecular oxygen is 2-phosphoglycolate.

U.S. Food and Drug Administration
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CFSAN/Office of Food Additive Safety
December 9, 2004

Agency Response Letter
GRAS Notice No. GRN 000152

Edward A. Steele
Vice President - Food, Dietary Supplement, & Cosmetic Consulting
AAC Consulting Group
7361 Calhoun Place
Suite 500
Rockville, MD 20855

Re: GRAS Notice No. GRN 000152

Dear Mr. Steele:

The Food and Drug Administration (FDA) is responding to the notice, dated June 3, 2004, that you submitted on behalf of Kraft Foods Global (Kraft) in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received this notice on June 9, 2004, filed it on June 15, 2004, and designated it as GRAS Notice No. GRN 000152.

The subject of the notice is sodium iron (III) ethylenediaminetetraacetate ($NaFe^{+3}EDTA$). For the purpose of this letter, we describe the subject of the notice as "sodium iron EDTA" (NaFeEDTA). The notice informs FDA of the view of Kraft that NaFeEDTA is GRAS, through scientific procedures, for use as a source of dietary iron for food fortification purposes in powdered meal replacement, flavored milk, and fruit-flavored beverages at a use level not to exceed 2.5 milligrams (mg) of iron per 200 milliliters (mL) of reconstituted beverage. Kraft intends NaFeEDTA for iron fortification in areas of the world with a high prevalence of iron deficiency.

As part of its notice, Kraft includes the report of a panel of individuals (Kraft's GRAS panel) who evaluated the data and information that are the basis for Kraft's GRAS determination. Kraft considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. Kraft's GRAS panel evaluated estimates of dietary exposure, the method of manufacture, specifications for the ingredient, and published studies on NaFeEDTA or its constituents (iron and EDTA). Kraft's GRAS panel concluded that NaFeEDTA meeting food-grade specifications is GRAS for its intended use.

Kraft describes the chemical identity of NaFeEDTA. NaFeEDTA (Chemical Abstracts Service

Registry Number 15708-41-05) exists in the trihydrate form, with the empirical formula $C_{10}H_{12}FeN_2NaO_8 \cdot 3H_2O$; it is also known by the chemical names sodium feredetate, sodium iron (III) EDTA, and sodium ferric EDTA. NaFeEDTA is a pale yellow powder that is very water-soluble at pH 7. Kraft notes that NaFeEDTA is relatively stable and is suitable for use in foods that are stored for long periods of time or prepared at high temperatures.

NaFeEDTA is prepared by reacting sodium EDTA with an iron chloride salt under acidic conditions, followed by filtering and drying, in accordance with current good manufacturing practice. The ingredient is manufactured to meet the Joint WHO/FAO Expert Committee on Food Additives (JECFA) food grade specifications, containing between 12.5 and 13.5 percent (by weight) iron and between 65.5 and 70.5 percent (by weight) EDTA. Purity specifications for NaFeEDTA include limits on lead, arsenic, and water-insoluble matter.

Kraft intends NaFeEDTA for iron fortification of powdered meal replacement, flavored milk, and fruit-flavored beverages in areas of the world with a high prevalence of iron deficiency. The intended level of fortification is 2.5 mg iron per 200 mL of reconstituted beverage; the beverage powder would contain 0.02 percent iron and 0.1 percent EDTA by weight. Reconstitution of 12.5 grams (g) of beverage powder with 200 mL of water would result in 2.5 mg iron per 200 mL of beverage, or a final concentration of 0.00125 percent iron (0.00125 g iron per 100 mL beverage).

Kraft estimates that the intended uses would result in a mean intake of 31.4 milligrams of NaFeEDTA per person per day (mg/p/d).[1] Kraft also estimates that the intended uses of NaFeEDTA would result in an iron intake of 4.2 mg/p/d at the mean and 8.2 mg/p/d at the 90th percentile and an EDTA intake of 21.8 mg/p/d at the mean and 43.0 mg/p/d at the 90th percentile.[2] Kraft provides estimates of intake for various age groups, including infants 0-2 years of age. Kraft notes that infants and children consume more food and drink than adults on a body weight basis, but states that realistic estimates of NaFeEDTA intake for young children correspond to an iron intake that remains within JECFA's recommended limit of intake for iron in supervised fortification programs (0.2 milligrams per kilogram of bodyweight per day (mg/kg bw/day).

Kraft notes that JECFA evaluated the use of NaFeEDTA as a nutritional supplement in foods in both 1993 and again in 1999. JECFA's provisional 1993 conclusion expressed concern about overfortification of NaFeEDTA and did not recommend its availability for general use by individuals. Consistent with this approach, Kraft intends to restrict the use of NaFeEDTA for iron fortification of powdered meal replacement, flavored milk, and fruit-flavored beverages to areas of the world with a high prevalence of iron deficiency.[3] JECFA's 1999 evaluation confirmed the provisional 1993 conclusion that "sodium iron EDTA could be considered safe for use in supervised food fortification programmes, when public health officials had determined the need for iron supplementation in the diet of a population," at iron intakes of approximately 0.2 mg/kg bw/d. Kraft notes that the restriction to use in supervised food fortification programs is included because the original request for evaluation of NaFeEDTA by JECFA from the Codex Committee for Food Additives and Contaminants was for this specific purpose alone.

Kraft describes results from studies that support the safety of NaFeEDTA for food fortification purposes. These studies include published human studies with NaFeEDTA, published and unpublished acute and subchronic (60-day, 90-day) animal toxicity studies with NaFeEDTA,

and a published mutagenicity study with NaFeEDTA. No adverse effects specific to NaFeEDTA were found. Kraft concludes from these studies that the safety of NaFeEDTA, as judged by the acute toxicity of the elemental iron content, is similar to that of ferrous sulfate. Kraft also concludes that the tissue distribution and deposition of iron is similar for orally-administered NaFeEDTA and ferrous sulfate.

Kraft cites generally available published documents on the safety of EDTA and its salts. Because the majority of NaFeEDTA dissociates in the gastrointestinal tract to produce free iron and EDTA, Kraft considers studies with calcium and sodium salts of EDTA to be relevant to the safety of NaFeEDTA. Kraft notes that extensive published studies in humans and other organisms have been conducted with EDTA and its salts, and that these studies have shown that EDTA is poorly absorbed and is essentially not metabolized. Kraft cites several published toxicity studies conducted with calcium disodium EDTA ($CaNa_2EDTA$) and trisodium EDTA (Na_3HEDTA), including studies reviewed by JECFA in its safety evaluations, and discusses some of these studies in detail. The notice also includes a discussion of a carcinogenicity study with Na_3HEDTA. In some rat studies, teratogenic effects were reported when EDTA was administered at very high doses (1000-1500 mg/kg bw/day) and zinc was restricted in the diet. No other adverse effects were observed in any of the studies. Kraft states that these studies contribute to the conclusion that NaFeEDTA is safe for its intended uses.

Iron Exposure

Kraft discusses the estimated contribution of NaFeEDTA to total dietary iron in populations where the ingredient would be used. Kraft provides examples of Latin American countries where iron deficiency anemia occurs in 30 to 70 percent of susceptible subpopulations (e.g., young children and women of childbearing age). Further, Kraft notes that iron intake in iron deficient populations, while not precisely known, may be assumed to be substantially less than the recommended dietary allowance (RDA) for iron.[4] Kraft estimates that the intended uses of NaFeEDTA would contribute an amount of iron equivalent to approximately 25 percent of the dietary intake of iron in those populations, assuming that typical iron intake is approximately 10 mg/p/d and one 200-mL serving (delivering 2.5 mg of iron) is consumed per day.

Kraft discusses the potential for iron overload from the intended uses of NaFeEDTA. Kraft cites published studies including long-term human studies in developing countries where NaFeEDTA was provided as an iron fortificant and a study of NaFeEDTA toxicity in rats. No adverse effects were seen in any of the studies, and Kraft notes that in the rat study the amount consumed was 50 times greater than the estimated 90th percentile intake of iron resulting from the intended uses of NaFeEDTA. Kraft states that these studies support its conclusion that the intended uses will not cause iron overload.

Kraft also discusses the levels of iron intake associated with acute toxicity in humans. Kraft notes that iron poisoning symptoms may occur from iron overload caused by acute ingestion of as little as 25 mg/kg bw/d of iron, with clinically significant iron poisoning occurring at iron doses of 60 mg/kg bw/d. Kraft estimates that a dose of 60 mg/kg bw/d of iron would be equivalent to consumption of approximately 100 liters (L) of reconstituted beverage containing 2.5 mg iron per 200 mL reconstituted soft drink. Kraft states that this estimate also supports the conclusion that the intended uses of NaFeEDTA will not cause toxicity in normal individuals.

Finally, Kraft discusses the intended uses of NaFeEDTA relative to the tolerable upper limit (UL) of intake for iron (45 mg/p/d for adults) established by the Institute of Medicine (IOM).[5] Kraft notes that the UL is based on gastrointestinal effects and considers that these effects constitute a self-limiting factor with respect to consumption of an iron-fortified beverage. Kraft further notes that an individual would need to consume 2.8 L of an iron-fortified beverage to reach the UL for iron, an amount that is well beyond the estimated intake from Kraft's intended uses. For iron deficient populations, where iron intake is presumed to be below the RDA, Kraft concludes that the risk of exceeding the UL is minimal given the intended uses of NaFeEDTA.

EDTA Exposure

Kraft discusses the contribution of NaFeEDTA to total dietary intake of EDTA in light of the JECFA acceptable daily intake (ADI) for EDTA of 2.5 mg/kg bw/d. Kraft states that EDTA intake resulting from the intended uses of NaFeEDTA would be 1.0 mg/kg/day of EDTA at the 90th percentile. Kraft notes that even if NaFeEDTA were used in populations already consuming EDTA-containing foods, the resulting total exposure to EDTA would not exceed the JECFA ADI for EDTA. In support of this statement, Kraft cites a published 1993 estimate of daily intake of EDTA in the U.S. from uses then currently regulated by FDA, where estimated daily intake (EDI) of EDTA was 0.22 mg/kg bw/day and 0.43 mg/kg bw/day at the 50th and 90th percentiles, respectively.[6]

Kraft discusses the potential for adverse effects of EDTA on the absorption of essential minerals in humans. Kraft acknowledges that, in large doses, EDTA may interfere with the absorption or retention of minerals but states that moderate doses of EDTA are generally considered to be innocuous. In support of this view, Kraft cites published long-term human studies with NaFeEDTA conducted in developing countries, published animal studies on EDTA and NaFeEDTA, and JECFA's evaluations of both EDTA and NaFeEDTA. Kraft concludes that the intended uses of NaFeEDTA would not interfere with the absorption of minerals such as calcium, copper, zinc and magnesium.

Potential Labeling Issues

Section 403(a) of the Federal Food, Drug, and Cosmetic Act (FFDCA) provides that a food shall be deemed to be misbranded if its labeling is false or misleading in any particular. Section 403(r) of the FFDCA lays out the statutory framework for health claims. In describing information that the notifier relies on to conclude that NaFeEDTA is GRAS under the conditions of its intended use, Kraft raises issues under these labeling provisions of the FFDCA. These issues include absorption of iron from dietary NaFeEDTA and its effects on iron status, and are the purview of the Office of Nutritional Products, Labeling and Dietary Supplements (ONPLDS) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety neither consulted with ONPLDS on these labeling issues nor evaluated the information in Kraft's notice to determine whether it would support any claims made about NaFeEDTA on the label or in labeling.

Conclusions

Based on the information provided by Kraft, as well as other information available to FDA, the agency has no questions at this time regarding Kraft's conclusion that NaFeEDTA is GRAS when used as a source of dietary iron for food fortification purposes in powdered meal

replacement, flavored milk, and fruit-flavored beverages in areas of the world with a high prevalence of iron deficiency. The agency has not, however, made its own determination regarding the GRAS status of the subject use of NaFeEDTA. As always, it is the continuing responsibility of Kraft to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,
/s/
Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

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(1)FDA estimates that NaFeEDTA exposure from Kraft's intended uses would be approximately 63 mg/p/d at the 90th percentile.

(2)FDA notes that Kraft's estimates, based on food consumption data from the USDA 1994-1996 Continuing Survey of Food Intake by Individuals (CSFII) and the 1998 Supplemental Children's Survey, may not be directly relevant to Kraft's target population with a high prevalence of iron deficiency. However, these estimates do provide some information on the level of exposure to be expected from use of NaFeEDTA in the specific food categories chosen by Kraft.

(3)Iron overload may be a greater problem in the United States than iron deficiency. FDA notes that the stated restriction on the intended uses of NaFeEDTA would minimize the risk of overfortification both within and outside the United States.

(4)The RDA for iron varies with age and gender, ranging from 6 mg/p/d for adult men 19-70+ years of age to 18 mg/p/d of iron for menstruating women 19-50 years of age. The RDAs for infants (7-12 months), children, adolescents, and teens are in this range. An RDA has not been set for infants 0-6 months of age.

(5)The UL for iron established by the IOM is 45 mg/p/d for adults (= 19 y) and adolescents (14-18 y) and 40 mg/p/d for infants (0-12 months) and children (1-13 y). FDA notes that, by definition, the UL represents the highest level of daily iron intake that is likely to pose no risk of adverse health effects in almost all individuals. Individuals with hereditary hemochromatosis, liver disease, or iron loading abnormalities are exceptionally sensitive to the effects of iron overload, and were not considered in the IOM's derivation of a UL for the general population.

[6]Calcium disodium EDTA ($CaNa_2EDTA$) and disodium EDTA (Na_2H_2EDTA) are approved as direct food additives under 21 CFR 172.120 and 21 CFR 172.135, respectively.

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FDA/Center for Food Safety & Applied Nutrition
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THIRD EDITION

SOILS AND SOIL FERTILITY

LOUIS M. THOMPSON
Associate Dean of Agriculture
Professor of Agronomy
Iowa State University

FREDERICK R. TROEH
Associate Professor of Agronomy
Iowa State University

McGRAW-HILL BOOK COMPANY
New York St. Louis San Francisco Düsseldorf Johannesburg
Kuala Lumpur London Mexico Montreal New Delhi
Panama Rio de Janeiro Singapore Sydney Toronto

Four of the six soils had less total pore space and less aeration pore space in the cultivated samples. The other two soils, Carrington and Marshall, showed slight increases in aeration pore space and no change in total pore space under cultivation. These two increases were considerably smaller and less significant than the large reductions in aeration pore space shown by some of the soils.

Practices that improve soil structure also help to improve aeration. The use of grass-legume meadows in a rotation helps to loosen the soil and to develop aeration porosity. Plowing at the right moisture level also loosens the soil, but this effect may disappear within a few weeks. Too much tillage speeds the decomposition of organic matter and reduces aggregate stability and porosity. The incorporation of large amounts of crop residues into the soil after each crop helps to maintain the organic-matter content of the soil and thereby favors both fertility and aeration.

Measuring soil porosity

Data such as those shown in Table 3-2 and represented in Figure 3-10 are generally obtained in two ways. The aeration porosity is measured by the tension method already described. Total porosity is calculated from data on bulk density and particle density.

Bulk Density The bulk density is the weight of the soil solids per unit volume of total soil. The pore space is a part of the volume of soil measured for bulk density, but the soil is oven-dried to drive the water out of the pores before the soil is weighed.

Direct measurement of bulk density requires both field and laboratory work. It is now possible to measure soil density in the field by means of gamma-ray attenuation. A radioactive source of γ-rays is lowered into a hole along with a detector. The source and the detector are arranged one above the other so that the γ-rays may reach the detector by passing along a curved path through the soil. Soil particles tend to absorb the γ-rays. The denser the soil, the more the γ-rays are absorbed and the smaller the count will be at the detector. The count can therefore be taken as an indication of soil bulk density. Allowance must be made in the interpretation for the water content of the soil, since water is excluded from the bulk density. Gamma-ray attenuation is therefore usually used along with a neutron probe to determine soil moisture content. The operation of the neutron probe is described in Chapter 4.

Bulk-density data are necessarily expressed in units of weight and volume. These can be any properly specified units (such as pounds per cubic foot), but the usual units are grams per cubic centimeter (g/cc). An older term, *volume weight*, was long used for this same property but is seldom seen in recent literature. Volume weight is determined by dividing the weight of a given volume of oven-dry soil by the weight of the same volume of water. Volume weight is

Table 3-3. Bulk Densities and Organic-matter Contents for Several Cultivated and Uncultivated Soils

Soil type	Bulk density		Organic matter, %*	
	Uncultivated	Cultivated	Uncultivated	Cultivated
Webster loam	0.91	1.14	10.9	8.4
Carrington silt loam	1.13	1.13	7.8	7.2
Ida silt	1.19	1.36	5.2	1.7
Marshall silt loam	1.08	1.08	5.5	3.8
Grundy silt loam	1.02	1.20	8.5	5.4
Edina silt loam	1.04	1.36	5.9	4.2

* Calculated by multiplying total nitrogen by 20.

Source: Anderson and Browning, 1949.

therefore a number without any units. This number is the same for all practical purposes as the bulk density expressed in grams per cubic centimeter.

The bulk density of the *A* horizons of mineral soils is usually between 1.0 and 1.6 g/cc (that of organic soils is lower and can be as low as 0.1 g/cc in sphagnum moss peats). The variation results mostly from differences in total pore space. As a general rule, the finer-textured soils have more pore space and lower bulk densities than sandy soils. Of course, the bulk density of any one soil varies according to its degree of compaction. Packing a soil decreases its pore space and increases its weight per unit volume. Overburden weight tends to compact the lower horizons and give them higher bulk densities than the A_1 horizons. A bulk density given without specifying the horizon is usually for an A_1 or an A_p horizon.

Organic matter decreases bulk density in two ways. First, organic matter is much lighter in weight than a corresponding volume of mineral matter; second, organic matter gives increased aggregate stability to a soil. The latter is by far the most important of the two effects in most soils, but both act to give lower bulk densities in soils with higher organic-matter contents. The bulk density usually increases when cultivation causes a loss in organic matter from a soil. Some of these changes are shown in Table 3-3. Cultivation reduced the organic-matter contents in all six of these soils and increased the bulk densities of most of them. The uncultivated Webster loam in this table illustrates how organic matter can reduce the bulk density even below the usual 1.0 to 1.6 g/cc range of mineral soils.

The relation between bulk density and the density of water is useful when it is desired to convert bulk density from grams per cubic centimeter to pounds per cubic foot or pounds per acre-foot (an area of one acre that is one foot deep) or per acre-furrow slice (the soil moved when one acre of land is plowed). The necessary constants are:

Density of water = 1.0 g/cc or 62.4 lb/cu ft
One acre = 43,560 sq ft

The weight of a cubic foot of soil can be calculated as follows:

$$\text{Lb/cu ft (soil)} = \text{g/cc (soil)} \times \frac{62.4 \text{ lb/cu ft (water)}}{1.0 \text{ g/cc (water)}}$$

A soil with a bulk density of 1.3 g/cc weighs:

$$1.3 \text{ g/cc} \times \frac{62.4 \text{ lb/cu ft}}{1.0 \text{ g/cc}} = 81.12 \text{ lb/cu ft}$$

The result should normally be rounded to 80 or 81 lb/cu ft.

The weight of an acre-foot of this soil can be calculated in the same manner by multiplying by the number of square feet in an acre (which equals the number of cubic feet in an acre-foot):

$$1.3 \times 62.4 \times 43{,}560 = 3{,}533{,}587.2 \text{ lb/acre-foot}$$

An acre-foot of soil is often assumed to weigh approximately 3.5 million pounds.

The weight of an acre-furrow slice can be calculated by correcting for its depth. For a 7-in. plow depth and a bulk density of 1.3 g/cc:

$$1.3 \times 62.4 \times 43{,}560 \times \frac{7}{12} = 2{,}061{,}259.2 \text{ lb/acre-furrow slice}$$

A slightly lower bulk density or a slightly shallower plow depth makes the result equal to 2 million lb, a figure that is frequently used as the approximate weight of an acre-furrow slice. This is an average weight; the actual weight for any particular soil may differ considerably from it. Nevertheless, the 2-million-lb value is quite useful because most analytical data are reported as parts per million (ppm). For example, a soil may contain 10 ppm of available phosphorus. This is equivalent to 20 parts per 2 million (pp2m) or 20 lb per acre-furrow slice. This simple multiplication by 2 can be applied to any data to be converted from ppm to approximate pounds per acre-furrow slice.

Particle Density Particle density is the other basic property that must be known to calculate total pore space. Particle density is the average density of the soil particles. The oven-dry weight of the soil is divided by the volume of the soil solids *excluding pore space*. The units used are almost always grams per cubic centimeter.

Much of the older literature uses the term *specific gravity* as a measure of the density of the soil particles. Like volume weight, it is determined by dividing by the weight of an equivalent volume of water and has no units. The specific gravity is numerically equal to the particle density in grams per cubic centimeter.

Iron deficiencies also can result from an excess of manganese and possibly from excess copper (Sommer, 1945). Manganese and copper can serve as oxidizing agents and convert ferrous iron to the more insoluble ferric form. Iron deficiencies caused by manganese toxicity occur in acid soils that otherwise would probably supply adequate iron for plant growth. Some areas where iron deficiencies have been identified are shown in Figure 14-2.

Iron in plants

Iron is absorbed by plants as the ferrous ion. The amount needed is not large. Stiebeling (1932) reported that the iron content of several fruits and vegetables ranged from less than 4 to more than 16 ppm. These amounts are approximately equal to the boron, manganese, and zinc contents of plants. Many plants contain more iron than this but not enough for iron to be considered a macronutrient.

Iron is necessary for the formation of chlorophyl and functions in some of the enzymes of the respiratory system (Schneider, Chesnin, and Jones, 1968). An iron deficiency results in the younger leaves being small and pale green or yellow in color. This shortage of chlorophyl is called *chlorosis*. The younger leaves are more affected than the older leaves because iron is relatively immobile inside the plant. Often the veins remain green while the areas between veins turn yellow from iron chlorosis.

Plants differ in their susceptibility to iron chlorosis. Soybeans become chlorotic on certain high-lime soils in Iowa (Figure 14-3) where corn, oats,

Figure 14-3 A soybean field in central Iowa showing iron chlorosis. The light-colored soybeans are yellow because the high-lime soil reduces the solubility of iron.

clover, and alfalfa show no chlorosis. Peach trees become chlorotic on the Houston clay soils in Texas, but cotton, corn, clover, and small grains grown on these soils show no chlorosis. Most plants obtain adequate iron for their needs from neutral or acid soils, but rhododendrons and azaleas are likely to become chlorotic if the soil pH is above 6.0.

Iron fertilizers

Ferrous sulfate has been used for many years as a treatment for iron deficiency. This salt is soluble in water and can be applied to either the plant or the soil as the circumstance demands. Florists sometimes include a small package of ferrous sulfate with azaleas or other potted plants which require appreciable quantities of soluble iron.

Application of ferrous sulfate to calcareous soils is generally ineffective. These soils already contain many times more iron than would ever be applied as a fertilizer. The problem is low availability resulting from the high pH. The added iron will soon react to form ferrous hydroxide or other very insoluble compounds like those already in the soil. Adding a form of soluble iron that can ionize and react in the soil is therefore not effective.

Foliar sprays of ferrous sulfate have proved quite effective when the iron requirement was not too high. A 1 percent solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) is recommended as a spray applied directly on the leaves of affected plants (Webb, 1965). The spray may be repeated in 7 to 10 days if needed.

In Florida, where almost half the citrus trees have been affected by lack of iron, the use of ferrous sulfate has not proved satisfactory. Most of the soils used for Florida citrus are acid, but other ions, especially copper, interfere with the availability of iron. An improved treatment of iron chlorosis using chelates was developed at the Florida Citrus Experiment Station at Lake Alfred. Applications of 10 to 20 g of actual iron per tree in a chelated form have been effective in correcting chlorosis for as long as 2 years. Chelates are discussed in the last section of this chapter.

Manganese

A small amount of manganese is essential, but a large amount is toxic to plants. Manganese can exist in several different oxidation states, but research indicates that most of the manganese in the soil solution is present as Mn^{++} (Geering, Hodgson, and Sdano, 1969). This form is also found in rocks. The Mn^{++} ion can fill the same role in mineral structures as Fe^{++} and Mg^{++} ions, but manganese is less abundant than either iron or magnesium.

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Microbial Degradation of Ethylenediaminetetraacetate in Soils and Sediments[1]

JAMES M. TIEDJE

Departments of Crop and Soil Sciences and of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824

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[^{14}C]ethylenediaminetetraacetate (EDTA) was shown to be slowly biodegraded to $^{14}CO_2$ in soils and sediments under aerobic conditions and by microorganisms in mixed liquid culture. EDTA chelates of Cu, Cd, Zn, Mn, Ca, and Fe added to soil were equally degraded, while Ni-EDTA was degraded more slowly.

Ethylenediaminetetraacetate (EDTA) is a synthetic organic chelant which is produced in large quantity for a wide variety of agricultural and industrial uses. Its eventual fate in the environment is not known, though it is commonly thought to be resistant to microbial degradation (1). Studies with EDTA incubated in sewage sludge have shown that the chelant does not support O_2 consumption (8) nor do its chelation properties diminish (4). However, by these methods low-level biodegradation over long periods could not be detected. In a preliminary report we provided evidence that [^{14}C]-EDTA was biodegraded to $^{14}CO_2$ in soils (J. M. Tiedje, E. S. Perry, and T. S. Savage, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, E29, p. 5). The present report provides additional evidence confirming microbial degradation of EDTA, as well as evidence for its biodegradation in mixed culture and in sediments and for degradation of heavy metal-EDTA chelates in soil.

Freshly collected soils and sediments were incubated in flasks with [^{14}C]EDTA at a concentration of 4.0 μg of free acid/g of soil. The [^{14}C]EDTA substrate solutions were prepared from the acid form of EDTA which was dissolved in deionized and glass-distilled water while neutralizing with NaOH; stock solutions were always stored in the dark at 2 C. The [^{14}C]carboxyl, acetate-2- and ethylene-1,2 (bridge)-labeled EDTA substrates were obtained from Mallinckrodt Chemical Co. Nuclear magnetic resonance analysis of [^{14}C]carboxyl-EDTA showed only absorptions expected of EDTA, indicating a chemical purity of at least 95%. Thin-layer chromatography and subsequent autoradiography showed >95% radiochemical purity of all three labeled compounds. The soils were common agricultural soils of mid-Michigan; their texture and pH are as follows: Spinks loamy fine sand, pH 6.4; Conover sandy loam, pH 7.4; and Miami sandy loam, pH 6.0. The incubation conditions and $^{14}CO_2$ analysis methodology were essentially as previously described (9). Photodegradation of EDTA, which has been demonstrated for Fe^{+3}-EDTA (6), was assumed not to be of significance since there was no difference in $^{14}CO_2$ evolution from flasks incubated in the dark (e.g., temperature study) compared to flasks incubated in room light. Anaerobic incubations were carried out in a glove box (2). The organic amendment consisted of equal quantities of glucose, glycine, acetate, and peptone added to soil in aqueous solutions to yield a final concentration of 0.4%. The mixed culture medium consisted of the same carbon substrates and 4.5 μg of [^{14}C]EDTA per ml in mineral salts (9); the inoculum was a water extract from soil.

The metal-EDTA chelates were made by mixing equimolar concentrations of the chloride salt of the metal and EDTA in aqueous solution. The pH of the solution was adjusted to neutrality before addition to soil. Sodium azide (2,000 μg/g) was added to several soils to approximate a sterile control. Sterile soils were obtained by autoclaving soil incubation flasks for 1 h on each of 3 successive days, after which filter-sterilized EDTA was aseptically added. Sterility was confirmed by the absence of turbidity in nutrient broth tubes inoculated with soil taken from the flasks after the termination of the experiment. Degradation is reported as both rate of $^{14}CO_2$ evolution, which was calculated from the best linear fit of plotted values over the

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5- to 8-week incubation period, and as total $^{14}CO_2$ evolved. The former value probably more truly represents EDTA degradation since this rate was more typical over the long term and among experiments and was not influenced by the more rapid but variable values often found for the initial period.

Extractable EDTA and metals were determined in the supernatant after centrifugation of an aqueous extract of soil. The metal concentration was determined by atomic absorption spectroscopy and the EDTA by scintillation counting. The ^{14}C in the aqueous extract was assumed to be primarily EDTA since thin-layer chromatography and autoradiography studies on other soils showed this compound to be the only significant labeled product (J. M. Tiedje, unpublished data), and other investigators (7) found this assumption to be consistent with the chelation properties observed.

Evidence showing that EDTA was degraded by microorganisms is summarized in Table 1. In soils sterilized (autoclaved) or treated with an inhibitor (azide), CO_2 production was minimal compared to untreated soils. The effect of temperature on rate of degradation shows a typical biological pattern with an increase up to 40 C but a sharp decline at 50 C. EDTA labeled in each of the three carbon positions shared approximately equal rates of CO_2 production, a finding not possible for solely chemical catalysis. A higher rate of degradation by surface soils than by subsoils is consistent with a higher distribution of microorganisms and organic matter in surface soils. The stimulation in degradation rate caused by the addition of readily degradable organic matter is also typical of a microbially catalyzed degradation. Finally, the mineralization of EDTA which coincided with growth of a mixed microbial population in a complex liquid medium in the absence of soil strongly indicates that microorganisms were responsible for EDTA metabolism. No volatilization of ^{14}C occurred in the absence of microorganisms. The correlation of degradation with high organic matter concentrations and the absence of successful enrichments with EDTA as a sole energy source suggest that co-metabolism is the mechanism of degradation. The conclusion that EDTA is degraded by microorganisms, albeit slowly, is consistent with a concurrent report of Belly et al. (3), who found EDTA degradation by organisms concentrated from an aerated lagoon which was receiving industrial effluent that contained EDTA.

The degradative abilities of the aerobically incubated sediments were similar to soil; the following rates of EDTA degradation (% per 4 weeks) and total degradation at 10 weeks (%), respectively, were found: Wintergreen Lake, 3.6 and 11.3; Clear Lake, 5.6 and 15.2; and Mill Pond, 3.2 and 9.1. No $^{14}CO_2$ production was observed under an

Heavy metal-ED result in inhibition in the case of Ni, inhibited 50% (Tab periments (not show and Brookston soils labels also showed EDTA metabolism. able and in a 1:1 rat and Cd were no previously associate Thus, mass action an tion reactions appa exchange so that a probably Fe- or Ca-E metabolism. The lo tabolism could be du ability of the cells to plex or to a lower conc is also interesting tha the only metal which vented the oxidation resting cells of an *Pseudomonas* species

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TABLE 1. *Summary of data indicating biodegradation of EDTA*

Treatment*	Soil	Rate of EDTA degradation (% per 4 wk)	Total $^{14}CO_2$ at 7 wk (%)
Autoclaved	Spinks	0.4	0.5
Azide	Spinks	0.6	
Anaerobic	Spinks	0.1	0.5
10 C	Spinks	0.8	2.5
20 C	Spinks	1.6	6.5
30 C	Spinks	4.8	12.0
40 C	Spinks	6.2	13.5
50 C	Spinks	2.2	4.5
Carboxyl	Spinks	4.6	17.0
Acetate-2	Spinks	4.8	23.0
Bridge	Spinks	4.4	14.5
Surface soil	Conover	6.7	16.5
Subsoil	Conover	0.8	2.5
Organic amendment	Miami	21.0[b]	28
Unamended	Miami	7.9 (4.8)[b]	15.5
Mixed liquid culture	No soil	13.6[c]	

* All incubation flasks contained 25 g of soil and 4 μg of [^{14}C]carboxyl EDTA per g and were incubated aerobically at 30 C unless otherwise indicated; data are means of duplicates.

[b] Calculated from the 2- to 3-week period; value in parentheses is for the comparable 5- to 8-week period.

[c] Calculated from the exponential phase of growth.

TABLE 2. *Metabolism and extractability of 1:1 metal-EDTA chelates after 18 days of incubation in Miami soil*

Metal	% of the original recovered*		
	$^{14}CO_2$	Metal	EDTA
Ni	10.8 a	61.4 a	61.5 b
Cu	20.2 b	0.0 c	51.5 cd
Cd	20.4 b	2.6 c	51.5 cd
Zn	19.8 b	12.4 bc	67.2 a
Mn	20.3 b	20.4 b	48.5 d
Ca	20.1 b		55.0 c
Fe	19.8 b		47.5 d
None	20.9 b		48.5 d

* Any two means within the same column followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test); data are means of four replicates.

indicating biodegradation
DTA

Rate of EDTA degradation (% per 4 wk)	Total $^{14}CO_2$ at 7 wk (%)
0.4	0.5
0.6	
0.1	0.5
0.8	2.5
1.6	6.5
4.8	12.0
6.2	13.5
2.2	4.5
4.6	17.0
4.8	23.0
4.4	14.5
6.7	16.5
0.8	2.5
21.0[a]	28
7.9 (4.8)[a]	15.5
13.6[c]	

ed 25 g of soil and 4 μg of
ere incubated aerobically at
: data are means of dupli-

eek period; value in paren-
-week period.
tial phase of growth.

extractability of 1:1
8 days of incubation in
oil

riginal recovered[a]

Metal	EDTA
61.4 a	61.5 b
0.0 c	51.5 cd
2.6 c	51.5 cd
12.4 bc	67.2 a
30.4 b	48.5 d
	55.0 c
	47.5 d
	48.5 d

same column followed
ficantly different at the
range test); data are

egradation (% per 4
on at 10 weeks (%),
intergreen Lake, 3.6
and 15.2; and Mill
O_2 production was

observed under anaerobic conditions.

Heavy metal-EDTA additions to soil did not result in inhibition of EDTA metabolism except in the case of Ni, for which metabolism was inhibited 50% (Table 2). Three additional experiments (not shown in Table 2) with Spinks and Brookston soils and carboxyl and bridge labels also showed only the Ni chelate to limit EDTA metabolism. Ni remained water extractable and in a 1:1 ratio with EDTA, whereas Cu and Cd were no longer extractable but the previously associated EDTA was (Table 2). Thus, mass action and competing soil precipitation reactions apparently resulted in ligand exchange so that a nontoxic EDTA substrate, probably Fe- or Ca-EDTA (7), was available for metabolism. The lower rate of Ni-EDTA metabolism could be due to Ni toxicity, to the inability of the cells to attack the Ni-EDTA complex or to a lower concentration of free EDTA. It is also interesting that Ni-nitrilotriacetate was the only metal which, at low concentrations, prevented the oxidation of nitrilotriacetate by resting cells of an nitrilotriacetate-degrading *Pseudomonas* species (5).

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at the soil-root interface and within the plant are all relevant considerations. It is not difficult to understand the effect of one metal in reducing the uptake of the other due to competition for uptake, as this is well documented (Gauch, 1972). Although Cd was present in the soil at much lower concentrations than Pb, it would likely be an effective competitor with Pb for uptake due to its greater soil "availability" (Lagerwerff, 1972). Also, Cd has been shown to reduce transpiration of corn leaves (Bazzaz et al., 1974), which may result in less translocation of Pb from the soil and roots to the shoots.

The reasons for the observed tendency for soil Pb to increase corn shoot Cd concentrations and uptake are less obvious and only speculative. One might speculate that Cd concentrations in the soil solution, and thus in the plant, are elevated by Pb as it has been shown that Pb more effectively competes for exchange sites on colloidal surfaces than Cd (Bittell and Miller, 1974). Previous experimentation with Pb-Cd interactions on growth and metal accumulation by 5-day-old corn plants indicated that both metals had a tendency to increase the other's concentration in the roots (Hassett et al., 1976). If that were the case in these experiments, it would be reasonable to expect greater Cd concentrations in the shoot due to Pb treatment, in that Cd is relatively mobile in the plant. One would not expect Pb concentrations to be similarly higher in the shoot, even though it may be in the roots, due to its known immobility in the plant. Unfortunately, metal analysis on root material were not obtained in the experiment since previous experience indicated serious errors due to surface contamination of the roots.

With the exception of Pb at the 10-day period, both Pb and Cd significantly (1% level) reduced the growth of corn shoots (Table 4). The negative interaction which was previously observed in the case of corn root elongation (Hassett et al., 1976) was not found with corn shoot growth. In fact, positive interactions (5% level) on shoot growth were observed at 17, 24, and 31 days. It might be inferred that the soil Cd reduction of Pb uptake is related to the apparent positive interaction on growth, except that shoot Cd was elevated by soil Pb. It is more likely that the explanation lies in the fact that plant growth was severely retarded by either of the metals singly, and the added burden of the other metal did not cause a growth inhibition comparable to its effects on a healthy plant. The response of plant growth to heavy metals is rarely a linear function of metal concentration, over an extended range of concentrations (Root et al., 1975). One might also speculate, in view of observed similarities in the mechanisms of physiological action of Pb and Cd (Bittell et al., 1974), that there is a competition between the two metals for physiologically active sites.

Table 4—The influence of Pb and Cd on the dry weight of corn shoots over time

Metal, Pb-Cd	Time period, days			
	10	17	24	31
—µg/g soil—	dry weight, g/plant†			
0-0	0.11	0.44	1.19	1.56
0-2.5	0.08	0.26	0.65	1.12
0-5	0.08	0.24	0.49	0.83
125-0	0.12	0.36	0.84	1.35
125-2.5	0.08	0.21	0.47	0.82
125-5	0.06	0.17	0.36	0.64
250-0	0.09	0.20	0.56	0.91
250-2.5	0.08	0.21	0.43	0.97
250-5	0.07	0.15	0.32	0.87
L.S.D. (0.01)	± 0.02 g	± 0.07 g	± 0.17 g	± 0.21 g

† Values are the means of three replicates.

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Influence of Environmental Parameters on EDTA Biodegradation in Soils and Sediments[1]

James M. Tiedje[2]

ABSTRACT

^{14}C-EDTA (ethylenediaminetraacetate) was slowly degraded to $^{14}CO_2$ by all soils tested from the major agricultural EDTA use areas, by soils varying in uses, pH, and texture; and by sediments from the Detroit River. EDTA degradation appears to be a result of cometabolism by established microbial populations. Production of $^{14}CO_2$ from EDTA occurred under aerobic but not anaerobic conditions. No detectable quantities of intermediates accumulated under either condition. EDTA degradation followed first-order kinetics for concentrations ranging from 0.4 to 90 ppm. Degradation was observed up to 1,000 ppm EDTA, the highest concentration tested. The date of sample collection had a marked effect on extent of degradation with the winter-collected samples showing greater than twice the degradation of summer-collected samples. The Q_{10} was two up to 30C. Initially degradation at 50C was limited, but after 9 weeks incubation the rate accelerated indicating the adaptation of thermo-tolerant EDTA-degrading populations. The extent of degradation among soils was variable; common values for 2 to 4 ppm of added ^{14}C-EDTA mineralized were 13 to 45% after 15 weeks and 65 to 70% after 45 weeks.

Additional Index Words: chelation, microbial, cometabolism

Large quantities of EDTA are produced for a variety of industrial, photographic, and agricultural uses including those as a micronutrient carrier, a stabilizer in manufactured products, and as a component in reaction mixtures for the production of a wide variety of products. Annual production of EDTA in the USA is in excess of 30 thousand metric tons (64.5 million pounds) (20). Because of the extent and diversity of use and the high water solubility of the chelant, it is probable that EDTA has entered many soil and water environments. EDTA had been thought to be resistant to microbial degradation (1) but recent reports by ourselves (16, 19) and others (3) conclude that EDTA can be degraded by natural microbial populations. Prior to these reports EDTA biodegradation by tomato (*Lycopersicon esculentum* Mill.) plants was reported (7, 8) though there is some question on whether photodegradation may have been involved. Degradation in animals, however, is minimal with most of the EDTA excreted (5, 14, 15). EDTA, particularly as the Fe(III) chelate, is known to be photodegraded (9, 10, 11), but this may not be a major factor in EDTA decomposition in most soil and water environments since, except for surfaces, little or no light is received.

Recently, intermediate products from EDTA degradation have been identified. Helly et al. (3), working with microbial populations from an aerated lagoon, detected the following possible intermediate products from NH_4Fe EDTA: ethylenediaminetriacetic acid (ED3A), iminodiacetic acid (IDA), *N,N*-ethylenediaminediacetic acid (*N,N*-EDDA), *N,N'*-ethylenediaminediacetic acid (*N,N'*-EDDA), ethylenediaminemonoacetic acid (EDMA), nitrilotriacetic acid (NTA), and glycine. Lockhart and Blakeley found the following photodegradation products of EDTA: CO, formaldehyde, ED3A, *N,N*-EDDA, EDDA, IDA, EDMA, and glycine. They found the chelate to be the most sensitive to Xenon light, and Co(II) to be somewhat sensitive and Na, Mg, Cu, Zn, Cd, and Hg chelates to be insensitive (11).

EDTA is readily leached in soil (4) and analcation-EDTA equilibria reactions suggest that EDT eventually predominate as the Fe(III) chelate in aci and as the Ca chelate in alkaline soils (4, 12). In a ous report (16), I have shown that EDTA added to complexes with Cu, Cd, Zn, Mn, Ca, or Fe was equal graded while Ni-EDTA was degraded more slowly. action appeared to provide a nontoxic EDTA substra degradation. EDTA would not be expected to be to microorganisms at the concentrations expected in n but it is known that certain bacteria, partic *Pseudomonas aeruginosa*, are relatively sensitive to E (6).

In this report I provide information on the ra EDTA biodegradation in a variety of soils and sedi and on the effect of temperature, anaerobiosis, E concentration, light, and date of sample collection o biodegradation. The absence of accumulation of i mediates of degradation in soils is also reported.

MATERIALS AND METHODS

Soil and Sediments

All soil samples were collected and stored in sealed plastic at 2C. Michigan samples were a composite of subsamples lected at three locations within a 10 m^2 area. Samples assayed effect of collection date on EDTA degradation (Table 2) were lected from the same site and assayed immediately after collect The Florida sample (Rockdale) was provided by Simon E. M Subtropical Experiment Station, Homestead, Florida, from a tr cal fruit growing area in which a variety of chelates had been u the sample consisted primarily of limestone. The Idaho sam (Portneuf) was provided by Larry Pennington, Simplot Soilbuildi Twin Falls, Idaho, from an irrigated agricultural area near the Sn River. The California sample (Yolo) was provided by Wayne M kelson, University of California, Davis, California, from a co and field bean (*Phaseolus vulgaris* L.) plot on the University perimental Farm.

The two Kansas samples (Naron) were provided by La Murphy, Kansas State University, Manhattan, Kansas, from a gr sorghum (*Sorghum bicolor* Moench.) experimental plot; one sam was from a plot to which 8.8 kg/ha Zn-EDTA had been added a the other from a plot with no EDTA applied. All non-Michig samples were freshly collected in May (except for the Kansas sa ple which was collected in July), shipped, and immediately assay for EDTA degradation. The Idaho, California, and Kansas samp were very dry when received. Surface soil samples (0–5 cm) we used unless otherwise indicated.

Sediments from the Livingstone, Trenton, and East Outer Che nels in the Detroit River-Lake Erie area were obtained by Peterse dredge and provided by Charles Elly, EPA, Grosse Ile, Michig The samples were freshly collected in March, maintained unde anaerobic conditions, and delivered for immediate aerobic an anaerobic assay of EDTA degradation.

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[2]Associate Professor of Soil Microbiology.

Flask Incubations

Twenty-five grams fresh weight of soil or sediment were added to 125-ml Erlenmeyer flasks that had been fitted with rubber stoppers which supported a 2-ml plastic beaker containing 1 ml of 1*N* NaOH. The soils were adjusted to approximately field capacity. Microdrops of neutralized (NaOH) ^{14}C-EDTA solutions (16) were randomly distributed over the soil surface using a 28-gage needle on a 100-µliter syringe. Generally 2.5 to 4.5 ppm EDTA (µg free acid/g soil) was the final soil concentration. All flasks were sealed and incubated at room temperature (25–27C) except in the temperature study where temperature-controlled incubators were used. The trapped $^{14}CO_2$ was assayed as previously described (18).

Anaerobic incubations were carried out in a glove box filled with 10% hydrogen and 90% nitrogen (2). Anaerobiosis was confirmed by the reduction of resazurin (−0.061 mV). Aqueous extracts of aerobic and anaerobic incubations were examined for metabolites by two-dimensional thin-layer chromatography and subsequent autoradiography using silica gel G plates (Analtech, Inc., Newark, Del.). The plates were developed in ethanol, NH_4OH (1:2) and ethanol, NH_4OH acetic acid (7:1:1) as described by Belly et al. (3) for the second dimension. For experiments in which the effect of EDTA concentration on degradation was determined, the following EDTA concentrations were used: 0.4, 1.0, 6.0, 12, 15, 30, 60, 90, 120, 200, 500, and 1,000 ppm.

To examine the effect of light on degradation, ^{14}C-acetate-2-EDTA was freshly dissolved in deionized, glass-distilled water, neutralized, and stored in an aluminum foil-wrapped flask at 2C. This substrate was added to eight flasks of Spinks soil, four of which were wrapped with aluminum foil and incubated in the dark, and the four remaining were incubated in the light on the lab bench. The maximum possible exposure of the EDTA to room light in the dark experiment was less than 5 min. $^{14}CO_2$ production was measured weekly for 7 weeks. After 4 weeks one light- and one dark-incubated flask were extracted with water, the extract concentrated and analyzed for ^{14}C components by TLC (thin-layer chromatography) and autoradiography. In a related experiment portions of an aqueous solution of the Fe(III) chelate of ^{14}C-carboxyl-EDTA were exposed to 5 hours of sunlight or 5 hours UV (ultra-violet) light (254 nm). The resulting photodegradation mixture as well as unexposed chelate were incubated in Spinks soil. Replicate flasks (four) for each of the above treatments were amended with a mixture of readily available carbon (0.1% glucose, 0.1% glycine, 0.1% acetate, and 0.1% peptone).

All of the reported data from the above experiments are means from duplicate or triplicate flasks except as noted. Generally the variation between duplicates was <8%. Controls for nonbiological transformations of EDTA (16) and for volatilization and trapping of $H^{14}CO_3^-$ from soil have been previously described (18). Data reported in % $^{14}CO_2$ refer to percentage of added ^{14}C recovered as $^{14}CO_2$.

EDTA

^{14}C-Carboxyl-EDTA, 2.54 mCi/*mM*, 97% purity (lot 4813) and ^{14}C-acetate-2-EDTA, 1.36 mCi/*mM*, 98% purity (lot 2938) were obtained from Mallinckrodt Chemical Co., St. Louis, Mo. Nuclear magnetic resonance (NMR) analysis of ^{14}C-carboxyl-EDTA showed only absorptions expected of EDTA indicating a chemical purity of at least 95%. Thin-layer chromatography, subsequent autoradiography, and extraction of the labeled spots showed a radiochemical purity of 99% for ^{14}C-carboxyl-EDTA and 95.2% for ^{14}C-acetate-2-EDTA. Nonradioactive EDTA, obtained from Baker Chemical Co., Phillipsburg, N. J. and Aldrich Chemical Co., Milwaukee, Wis. was used to dilute the ^{14}C-EDTA in experiments where >12 ppm EDTA was used. No differences in degradation rate could be observed among different sources or ratios of labeled and unlabeled EDTA (1:9 and 1:3). Fe(III) EDTA was made by mixing equivalent molar ratios of EDTA and $FeCl_3$.

RESULTS

EDTA was biodegraded in a variety of soils of different geographical origin, texture, use, and pH as is shown in Table 1. Degradation was minimal in subsoil samples. The soils from Florida, Idaho, California, and Kansas were representative of the agricultural soils in the major EDTA use areas. The Florida soil had one of the highest degradation rates observed. The two Kansas samples, one of which was previously treated with Zn-EDTA, showed similar degradation rates. Long term incubation of EDTA with soil (45 weeks) showed 65–70% of the label recovered as $^{14}CO_2$.

Concentrations of EDTA ranging from 0.4 to 120 ppm generally gave the pattern of $^{14}CO_2$ evolution depicted in Fig. 1A for 30 ppm. The higher concentrations, 500 and 1,000 ppm, were initially inhibitory but eventually stimulated degradation. When the velocity of degradation was determined from the linear portion of the degradation curves (7 to 17 weeks) the rate of carboxyl mineralization appeared to be first order up to a concentration of 90 ppm (Fig. 1B). The lower velocities for 120 and 200 ppm suggest that the enzyme(s) responsible for degradation may be approaching saturation. The relatively high concentration needed for such an effect suggests a low affinity of substrate to enzyme. The velocities of degradation for concentrations of 500 and 1,000 ppm were greater than first order which indicates microbial growth. This explanation is also supported by the shape of the 1,000 ppm curve (Fig. 1A) which is typical of bacterial growth.

Table 1—Biodegradation of ^{14}C-carboxyl-EDTA (~4 ppm) in a variety of soils

Sample source	Soil	Use	pH (H_2O)	Rate of EDTA degradation†	Total $^{14}CO_2$	
				%/4 weeks	15 weeks	45 weeks
					%	
Michigan‡	Houghton muck	Vegetables	5.9	2.6	10.5	
	Houghton muck subsoil	"	6.1	0.8	3.5	
	Miami sandy loam (s)	Pasture	6.0	10.0	44.5	
	Brookston loam (l)	Forest	6.8	11.0	43.5	65.0
	Conover sl	Soybeans	6.7	12.2	46.0	70.5
	Spinks loamy fine sand (lfs)	Corn	6.4	11.3	32.0	
	Spinks lfs-subsoil	"	6.3	0.6	3.5	
	Spinks sl	Forest	4.6	"	19.0	
	Conover sl	Corn	7.4	6.7	27.5	
	Conover sl-subsoil	"	7.6	0.8	4.0	
Florida	Rockdale	Tropical fruits	7.9	9.0	34.0	
Idaho	Portneuf sicl	Sugar beets	7.9	1.8	9.5	
California	Yolo l	Cereals/beans	7.6	3.0	13.0	
Kansas	Naron l	Sorghum	5.9	1.9(4.2)§	13.0	

† Rate calculated from best linear fit of plotted values in the 5–8 week incubation period.
‡ Samples collected in February.
§ Accelerated rate of degradation which began at 10 weeks of incubation.

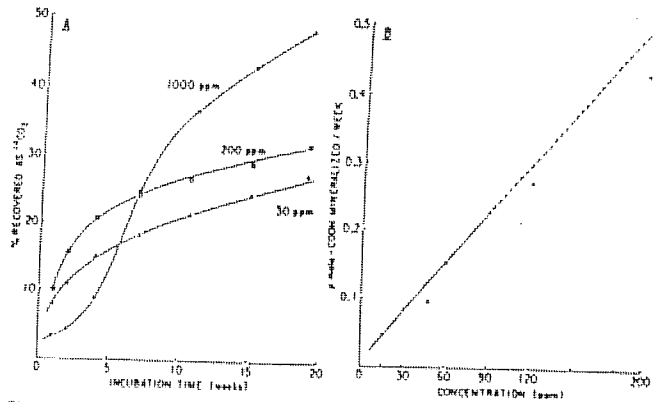

Fig. 1—*(A)* Effect of EDTA concentration on biodegradation of ^{14}C-carboxyl-EDTA in Spinks soil. *(B)* Velocity of degradation as fu of concentration.

An increase in temperature of incubation from 10 to 30C resulted in an increase in rate of degradation (Fig. 2); the Q_{10} was approximately two. Incubation at 50C initially showed a much lower rate of degradation; however, at 9 weeks an accelerated degradation rate began which exceeded the rate for all other temperatures studied. This pattern of metabolism suggests that the catabolic enzymes were initially inactivated by the higher temperatures, as expected for an indigenous soil population, but that a thermotolerant population was eventually selected which catalyzed the EDTA degradation.

EDTA degradation in aerobic soils or anaerobic sediments (Fig. 3) under anaerobic conditions did not result in $^{14}CO_2$ production. This finding in itself does not confirm the absence of anaerobic degradation since fermenta-

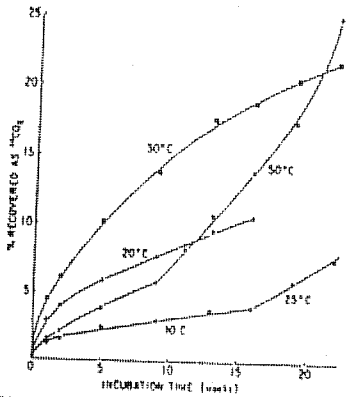

Fig. 2—Effect of incubation temperature on biodegradation of ^{14}C-carboxyl-EDTA (2.8 ppm) in Spinks soil. The flasks at 10C were shifted to 25C at 16 weeks.

tion would not necessarily yield $^{14}CO_2$ but rather produce fermentation end products. Such products, ever, would be expected to be rapidly metabolize $^{14}CO_2$ under aerobic conditions. After periods of an bic incubation subsequent aerobic metabolism did no sult in a rate of $^{14}CO_2$ production in excess of that fo for continuous aerobic metabolism (Fig. 3). Thus, EI does not appear to be subject to significant anaerobic gradation. Also, in support of this conclusion is the sence of any ^{14}C-products, as determined by thin-la chromatography, in the aqueous extract from Spinks which had been incubated anaerobically for 14 weeks; recovery of added label in the extract was 97.7%.

The date of collection of the soil sample had a ma effect on the rate of EDTA degradation when assa under similar temperature and moisture conditions (Ta 2). The samples collected in the winter when the soil frozen yielded the highest rates of degradation and th collected in the summer produced the lowest.

The addition of a large quantity (1,000 ppm) of ED to soil resulted in an even greater rate of total CO_2 respi tion than could be derived from complete mineralizati

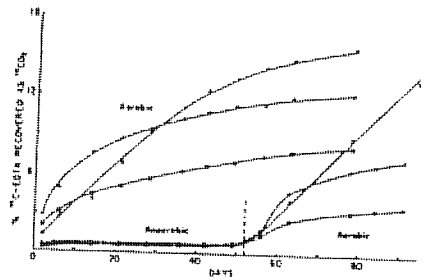

Fig. 3—Biodegradation of ^{14}C-carboxyl-EDTA (4.4 ppm) under aerobic and anaerobic-to-aerobic conditions in sediments from the Detroit River-Lake Erie area: Livingstone Channel (O) Trenton Channel (Δ), and East Outer Channel (□).

Table 2—Effect of date of sample collection on biodegradation of ^{14}C-carboxyl-EDTA (2.8 ppm) by four soils

Collection date	Total $^{14}CO_2$ evolved in 30 weeks			
	Miami	Spinks	Conover	Brookston
	% of ^{14}C-EDTA recovered as $^{14}CO_2$			
February 1974†	38	31	36	35
April 1974	24	24	14	16
June 1974	17	20	14	17
September 1974	11	14	13	13
January 1975†	30	18	24	–

† Soils collected frozen, thawed, and immediately incubated with EDTA.

of the added EDTA. This suggests that the EDTA, because of its chelating action, was increasing the availability of soil organic matter for microbial attack. After two weeks, however, the rate of respiration returned to the rate found in unamended soil. A portion of the CO_2 respired was presumably from the EDTA molecule itself but, because of the general stimulation to soil respiration, it was not possible to discern the extent of chelate degradation by total CO_2 evolution. The experiment does, however, show that high concentrations of EDTA are not inhibitory to soil respiration.

In all experiments the initial quantity of $^{14}CO_2$ produced was higher than would be predicted from the subsequent pattern of degradation. Since Fe(III) EDTA is sensitive to photolysis and this would be predicted as the predominant ion species in most soils, possible photodegradation was examined. As shown in Table 3, the presence or absence of room light had little effect on $^{14}CO_2$ production from ^{14}C-acetate-2-EDTA, but the initial high values were present under both conditions. Though photodegradation did not account for this initial pulse, the sensitivity of Fe(III) EDTA to sunlight and UV light is apparent since 60.9% and 15.1%, respectively, of the carboxyl label was mineralized by these treatments. When the remaining EDTA and photolysis products were incubated with soil, initial rapid mineralization occurred, particularly when no organic matter was added (Table 3). This suggests that certain components of the photolysis mixture are more readily degradable than EDTA. Glycine, IDA, and possibly EDMA and EDDA are examples of reported photolytic products which would be more readily degraded. In the case of the sunlight pretreatment, approximately 86.5% of the carboxyl groups were volatilized by the combined photo and biocatalysis. Also, as shown in Table 3, the addition of organic matter caused a delay

Table 3—Effect of room light, photolysis, and organic matter (OM) additions on degradation of ^{14}C-acetate-2-EDTA and ^{14}C-carboxyl-EDTA

(Fe) ^{14}C-acetate-2-EDTA			[Fe(III)] ^{14}C-carboxyl-EDTA					
Incubation conditions			Pretreatment of EDTA					
			None		UV†		Sunlight†	
Incubation time	Light, soil	Dark, soil	Dark soil + OM	soil	soil + OM	soil	soil + OM	soil	soil + OM
weeks	% of ^{14}C added recovered as $^{14}CO_2$								
0.5	6.4	6.6	1.0	3.6	1.7	16.1	7.1	50.6	38.1
1	7.5	7.0	2.5	4.6	2.3	19.7	12.6	54.9	60.0
4	12.3	11.9	9.4	12.9	8.5	32.2	34.7	65.6	64.6
7	16.8	15.7	13.1	–	–	–	–	–	–

† Percent of label lost during pretreatment of 8 hours in sunlight was 60.9% and of 6 hours in UV light was 15.1%.

in the mineralization of EDTA. Apparently, the microbial populations used the more readily available carbon before attacking EDTA and its photolysis products.

Significant quantities of intermediates of EDTA degradation did not accumulate in soils under either light or dark incubations. From the autoradiogram shown in Fig. 4, it is apparent that ^{14}C-EDTA is the major labeled compound; it accounts for 98.1% of the label on the chromatogram. Likely intermediates of EDTA degradation such as ED3A, *N,N*-EDDA, *N,N'*-EDDA, and EDMA separated from EDTA in these TLC solvent systems.

DISCUSSION

The surface soils and sediments examined represent a variety of geographic areas, uses, pH, and textures. Since all showed EDTA biodegradation, this capacity appears to be rather common in nature. My previous suggestion that EDTA degradation is a result of cometabolism by established populations (16) is further supported by finding that the pattern of degradation does not reveal any lag-to-acceleration phase, such as occurred for nitrilotriacetate (18), which would be expected if an organism could utilize EDTA as a carbon source for growth. Also,

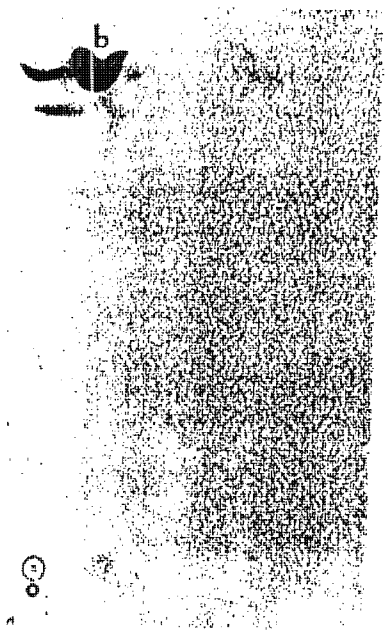

Fig. 4—Two-dimensional autoradiogram of ^{14}C-labeled components found in water extract of Spinks soil which had been incubated 4 weeks with ^{14}C-acetate-2-EDTA. The spot labeled "b" is EDTA which accounted for 98.1% of the label on the TLC plate. The sample origin is indicated by "O."

the correlation of activity with depth and thus organic matter and population density and the apparent low affinity of the substrate for the enzyme(s) support cometabolism as the mechanism. Not all organisms can participate in EDTA degradation since a *Pseudomonas* sp. growing on the closely related tertiary amine chelant, NTA, could not metabolize EDTA (J. M. Tiedje, unpublished data).

Though a temporary accumulation of EDTA intermediates, particularly ED3A and IDA, was found with lagoon microorganisms (3), no such accumulation occurred in soils incubated under aerobic, anaerobic, light or dark conditions. The absence of intermediates in soils agrees with the previous finding of equal rates of degradation to $^{14}CO_2$ of ^{14}C-ethylene(bridge)-EDTA and ^{14}C-acetate- or carboxyl-EDTA (16). Thus, the initial degradative step appears to be the rate-limiting step in EDTA biodegradation.

Anaerobic degradation of EDTA to CO_2 or to a pool of anaerobic end products did not occur in soils or sediments, the latter having dominant natural anaerobic populations. However, given a cometabolic mechanism, which may be widely distributed among populations, it would be surprising if EDTA degradation was totally absent from anaerobic populations unless the degradation pathway required molecular oxygen, as is the case for NTA metabolism (17).

A surprisingly important factor affecting the rate of EDTA degradation was the date of sample collection. Winter-collected samples showed over twice the degradation rate of the summer-collected samples. It is well known that after periods of freezing and thawing new organic matter, from lysis and physical rearrangement of organo-mineral matrices, becomes available for microbial attack. Thus, this available organic matter appears to stimulate populations capable of EDTA degradation. This finding of the major effect of collection date does impart greater difficulty to predicting rates of metabolism of EDTA or any other cometabolizable substrate in nature, since the nutritional "state" of the natural system must be taken into account.

Extended incubation of EDTA in soil (45 weeks) showed 65–70% of the EDTA label recovered as $^{14}CO_2$. The actual EDTA dissipation could be greater since significant quantities of the ^{14}C should have been incorporated into cellular constituents and resistant soil humus. Studies reviewed by Payne (13) have shown the ratio of carbon assimilated to carbon respired to be 3:2 for several substrates and organisms. This explanation is consistent with our previous finding that only 50–60% of the EDTA could be recovered in aqueous extracts of soil while 20% was recovered as $^{14}CO_2$ (16). The unrecovered label (20–30%) could have been assimilated, although binding of EDTA to soil does occur (12) and could also contribute to this loss of label. Binding to soil did not occur under anaerobic conditions since 97.7% of the label was water extractable.

The time-course of EDTA degradation in flask experiments can be divdied into two major phases—the initial high rate and the rate beginning at 4 to 6 weeks, often fairly stable for 10 to 15 weeks, before declining. Part of the cause for this high initial rate can be explained by

labeled contaminants in the ^{14}C-EDTA. Photoche degradation, however, did not contribute to this val is possible that a mineral catalysis, possibly asso with the aerobic binding of EDTA to soil, may occu contribute to this initial degradation. Because of th certainty of the mechanism for and origin of this i rate, the data have been expressed as rates for th week period, since they did not appear to be influe by this initial pulse. The values reported should be sidered as approximations, most useful for relative parisons among samples and for establishing trends, should not be considered precise, predictable rates minor unexplained differences did occur among com ble experiments; replication within experiments, how was good.

These findings indicate that EDTA should be bi graded in most aerobic soils and sediments provided ficient organic matter is available to support a ge microbial population and the residence time of EDT such environments is lengthy.

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Phosphates in Soils Treated with Sewage Water: III. Kinetic Studies on the Reaction of Phosphate with Aluminum Compounds[1]

W. H. van Riemsdijk, F. A. Weststrate, and J. Beek[2]

ABSTRACT

The reaction of phosphate with aluminumhydroxide and αAl_2O_3 was studied at an initial phosphate concentration level of 0.35 mmoles/liter and a temperature of 20C. Solutions of KH_2PO_4 and synthetic inorganic sewage water were used as phosphate sources. In all experiments involved, a marked change of the reaction rate was found after about 1 day in the case of $Al(OH)_3$ and about 4 days in the case of αAl_2O_3. This can be explained by the assumption that at these points the formation of a solid aluminum-phosphate is initiated. This new phase was later identified as Sterrettite by means of electronmicroscopy, electron diffraction, and X-ray diffraction.

Additional Index Words: adsorption, precipitation, phosphate fixation, crystalline aluminum phosphate.

Long term disposal of raw sewage water on a sandy soil resulted in the accumulation of phosphates in the soil profile. With respect to the mechanism of the phosphate retention by the soil system it was suggested by Beek and de Haan (1973) that precipitation reactions may play an important role in addition to adsorptive bonding on soil constituents.

Careful fractionation studies (Beek et al., 1977, compare part II of this contribution) indicated that the accumulated phosphates are most likely associated with aluminum. In view of these findings the reaction of phosphate with X-ray amorphous $Al(OH)_3$ and αAl_2O_3 was studied. The main objective of this study was to investigate the possible formation of a new solid phosphate phase under the experimental conditions and to identify such a new phase. The kinetics of such precipitation reactions constitute a prerequisite for the development of mathematical models for the description of phosphate behavior in soils intermittently treated with large amounts of sewage water. The system under study here, thus, is a model system, which may give insight in the reaction mechanisms involved. Similar experiments with the soil of the sewage farm are now in progress.

In the experiments use was made of synthetic sewage water as a phosphate source. For comparison, similar experiments were performed using a pure KH_2PO_4 solution as the phosphate source. Results of these latter experiments have been published in part elsewhere (van Riemsdijk et al., 1975).

MATERIALS AND METHODS

Using a ball mill, αAl_2O_3 (Merck, Germany) was ground. Following separation by wet sieving, the fraction < 50 μm was used in the experiments. Preparation of $Al(OH)_3$ was performed according to Hsu and Rennie (1962). The amorphous state of the reaction product was confirmed by X-ray diffraction. Both adsorbents were subjected to specific surface area measurements with a Perkin-Elmer Model 212-e Sorptometer, using nitrogen gas. In the calculation of the specific surface area use was made of the BET equation. Values for specific surface area were found as 3.8 m^2/g for αAl_2O_3 and 226 m^2/g for $Al(OH)_3$.

The adsorbents were suspended in solutions which contained all main inorganic ions of the sewage water at the same average concentration levels as present in the waste water used on the sewage farm. This is with the exception that HCO_3^- was substituted by Cl^- and that silicate was excluded. Replacement of HCO_3^- by Cl^- was done because it is impossible to obtain the same pH in the synthetic sewage water as in the raw sewage water if both contain the same amount of HCO_3^-. This is caused by the fact that the sewage water is oversaturated with CO_2 with respect to equilibrium with the atmosphere. Solution compositions are presented in Table 1. In addition, solutions of 0.35 mmoles/liter KH_2PO_4 (pH 5) were used. The concentration levels of Table 1 represent initial values at the start of the adsorption experiments. Preparation of the solutions was performed at twice these initial reaction concentrations, as the solutions were mixed 1:1 with suspensions of both adsorbents involved.

Suspensions of αAl_2O_3 and $Al(OH)_3$ were made as given in Table 2. The pH was adjusted within 0.1 pH unit by using NaOH and HCl, and was constant after approximately 1 week.

Table 1—Composition of the inorganic synthetic sewage water medium (SSW medium)

	pH 5 (SSW 5)	pH 6 (SSW 6)
	mmoles/liter	
$CaSO_4.2H_2O$	0.84	0.84
$CaCl_2.2H_2O$	0.94	0.93
NH_4Cl	1.86	1.87
$MgCl_2$	0.34	0.35
KNO_3	0.19	0.19
NaF	0.01	0.01
KCl	0.34	0.17
KOH	–	0.16
$NaCl$	6.25	6.29
KH_2PO_4	0.35	0.35

[1]Contribution from the Lab. of Soils and Fert., Agric. State Univ., 3 De Dreyen, Wageningen, the Netherlands. Support in part by the Comm. on Manure and Odour Problems in Animal Husbandry is gratefully acknowledged. Received 6 Nov. 1975.
[2]Chemist, Graduate Student, and Soil Scientist, respectively.

Table 2—Aluminum suspensions

Suspension	Solid concentration	pH
	g/0.5 liter	
Al_2O_3	2.5	5
Al_2O_3	2.5	6
$Al(OH)_3$	0.1	5
$Al(OH)_3$	0.1	6

After addition of the various solutions the suspensions were continuously mixed by means of an end-over-end shaker at a temperature of 20.5 ± 0.1C. The pH of the suspensions was measured with a combined calomel reference-glass electrode in unstirred suspensions; these pH measurements were performed after a sedimentation period of 20 min. During the experiments the pH was kept constant by addition of HCl.

Phosphate concentrations were measured according to the method of Murphy and Riley (1962) following filtration through a 0.1 μm membrane filter.

Samples used for X-ray diffraction were filtered after a reaction time period of 62 days. Following washing with distilled water and vacuum drying, these samples were analyzed with a Philips diffractometer using Co-Kα radiation. A Philips model EM 301 electron microscope was employed in the electron diffraction measurements.

RESULTS

The experimental results are presented graphically in Fig. 1 and 2. Figure 1 shows the course of the phosphate solution concentration as a function of reaction time.

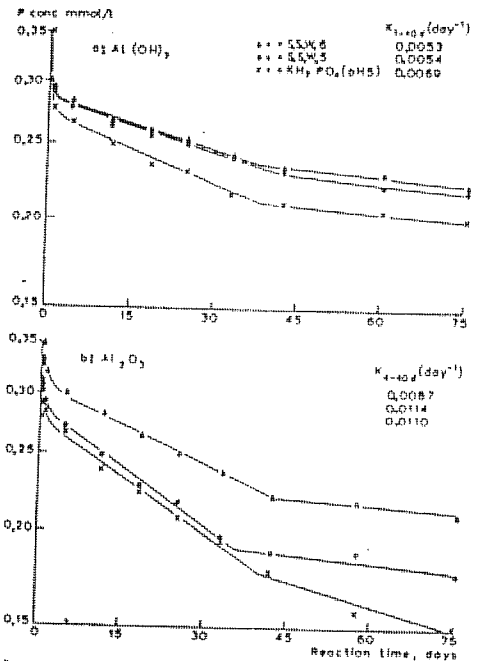

Fig. 1—A semilog plot of the measured phosphate concentration as a function of reaction time.

Figure 1a refers to the $Al(OH)_3$ system, indicating for the time period from about 1 day until about 40 after the start of the experiment the rate of the reac can be described by first order kinetics according to:

$$\ln \frac{C_0}{C_t} = K' \cdot t$$

where C_t is the phosphate concentration at time t, C_0 the phosphate concentration at about 1 day reaction ti K' is the rate constant (in day^{-1}), and t is the reaction ti (in day). The rate constant (K') depends on the magnitu of the surface area available for adsorption and on the of the system.

Results for the αAl_2O_3 system are given in Fig. 1b. that case, the first order reaction starts after a reacti time of about 4 days. Chen et al. (1973) have report similar observations for the reaction of phosphate wi αAl_2O_3 and with kaolinite.

When these same experimental data are expressed terms of amount of phosphate removed from the soluti per gram of adsorbent and plotted on a double log sca as a function of time, two straight intersecting lines a found for each reaction system as indicated by Fig. The rate of phosphate removal increases considerably about 1 day and 4 days reaction time for $Al(OH)_3$ an

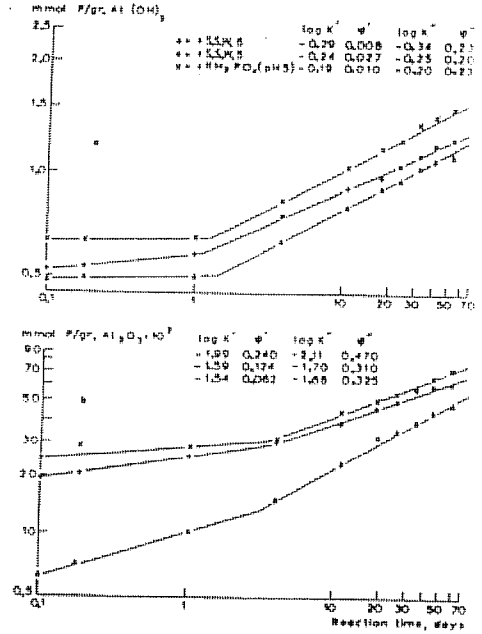

Fig. 2—A double log plot of the amount of phosphate removed as a function of reaction time. Expressing the lines shown as $\log X = \log K' + \phi \log t$, where X = phosphate removed (mmoles/g), K' and ϕ' are the constants for the initial straight line, and K'' and ϕ'' dito for the second part of the reaction.

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Toxicological Profile, Current Use, and Regulatory Issues on EDTA Compounds for Assessing Use of Sodium Iron EDTA for Food Fortification

PAUL WHITTAKER, JOHN E. VANDERVEEN, MICHAEL J. DINOVI, PAUL M. KUZNESOF, AND VIRGINIA C. DUNKEL

Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC 20204

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The U.S. Food and Drug Administration (FDA) has approved the use of disodium and calcium disodium ethylenediaminetetraacetate (EDTA) for direct addition to food. The international nutrition community is interested in obtaining approval for the use of sodium iron(III) EDTA (NaFeEDTA) for dietary iron fortification because of its high iron bioavailability, its enhancement of intrinsic food iron uptake, and its stability under storage and food preparation conditions. A major concern in the United States has been the presumed extensive use of EDTA in the American food supply. Recently, an update of the estimated exposure to EDTA suggests that the exposure is much lower than previously assumed. This reduction may allow new uses of NaFeEDTA in food. The following issues are discussed in relation to the possible use of NaFeEDTA: toxicological profile of EDTA compounds, acceptable daily intake of EDTA, and estimated daily intake of EDTA in the United States.

INTRODUCTION

The international nutrition community is interested in using sodium iron(III) ethylenediaminetetraacetate (NaFeEDTA) for dietary iron fortification. Clinical trials in Guatemala, Venezuela, Thailand, and South Africa have demonstrated that NaFeEDTA successfully reduces iron deficiency anemia (Ballot *et al.*, 1989; Garby and Areekul, 1974; Martinez-Torres *et al.*, 1979; Viteri *et al.*, 1978), and several countries have already used NaFeEDTA for food fortification. Many countries would like NaFeEDTA to be approved for use in the United States along with EDTA compounds already regulated. These countries rely on approval decisions on food additives made by the United States, largely because of their own limited resources. There are several reasons for pursuing the use of NaFeEDTA for iron fortification: (1) It has a relatively high iron bioavailability, which is generally not reduced by dietary inhibitors (Viteri *et al.*, 1978); (2) it is a fairly stable compound (MacPhail *et al.*, 1985; Sawyer and McKinnie, 1960); and (3) it usually does not cause undesirable characteristics such as rancidity and discoloration of food (MacPhail *et al.*, 1985). A major concern has

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been the presumed extensive use of EDTA in the American food supply; however, a recent update shows that exposure to EDTA is much lower than previously estimated. This reduced exposure would allow new uses of NaFeEDTA in food since physiochemical considerations indicate that NaFeEDTA would have a toxicological profile analogous to currently regulated EDTA salts. Outside the United States, where the number of processed foods consumed and hence the dietary intake of EDTA is low, it would be reasonable to consider using NaFeEDTA. A remaining concern with increasing the use of EDTA is its possible negative effects on the zinc status of the population.

In the United States, EDTA compounds are used in foods as chelating agents to bind trace metal ions, which may produce undesirable effects on the color, clarity, and stability of foods. EDTA is added to foods as a preservative, processing aid, and color stabilizer. The chelation of metal ions by EDTA is nonspecific and depends in part on the relative stabilities of the individual metal–EDTA chelates. In solution, metal ions with higher affinities displace metallic ions with lower affinities from the EDTA complex. The most common undesirable ions are iron (Fe^{3+}) and copper (Cu^{2+}), which can catalyze the oxidation of vitamins and unsaturated fats and oils. EDTA in the range of 25–800 parts per million may be commonly added to foods such as canned carbonated soft drinks, canned cooked vegetables, potato salad, frozen white potatoes, mayonnaise, salad dressings, margarine, sandwich spreads, and canned cooked shellfish (FDA, 1992b,c).

FDA has approved the use of EDTA and several of its salts as direct (FDA, 1992a) and indirect (FDA, 1992d) food additives (FDA, 1992b,c,e). However, only disodium EDTA (Na_2EDTA) and calcium disodium EDTA ($CaNa_2EDTA$), which constitute most of the EDTA salts in the American food supply, have been approved as direct additives to foods. The most recent survey by the National Academy of Sciences (NAS) (Table 1) shows that 24,100 pounds of Na_2EDTA and 162,000 pounds of $CaNa_2EDTA$ were available to be added to the American food supply in 1987 (NAS, 1989). Other approved EDTA compounds, indirectly added to food either singly or mixed, are calcium, iron(III), potassium, or sodium salts of EDTA (FDA, 1992e). The present report discusses the toxicological profile of EDTA compounds, the acceptable daily intake (ADI) for EDTA, and the estimated daily intake (EDI) for EDTA in the United States.

TOXICOLOGICAL PROFILE OF EDTA

In the toxicological evaluation of EDTA, both Na_2EDTA and $CaNa_2EDTA$ have been used to assess chronic toxicity, carcinogenicity, genotoxicity, and teratogenicity. NaFeEDTA has not been tested, but chemical properties of EDTA complexes indicate that the EDTA from NaFeEDTA would function in a similar way to EDTA from regulated salt complexes.

Chronic Toxicity

Oser *et al.* (1963) reported the results of a chronic feeding study in FDRL rats fed $CaNa_2EDTA$ in the diet at levels of 0, 50, 125, and 250 mg/kg body wt/day. Reproduction, lactation, and the long-term effects of $CaNa_2EDTA$ in the diet were assessed

TABLE I

AMOUNT OF EDTA IN THE U.S. FOOD SUPPLY FOR THE 4 YEARS SURVEYED BY THE NATIONAL ACADEMY OF SCIENCES SURVEYS[a]

	Pounds			
EDTA compound	1970	1976	1982	1987
$Na_2EDTA \cdot 2H_2O$	15,900	24,600	166,000[b]	24,100
$CaNa_2EDTA \cdot 2H_2O$	75,000	110,000	148,000	162,000

[a] NAS, 1989.
[b] Value appears to be an outlier.

through four successive generations. Terminal observations were made on animals receiving the diets for 0.5, 1.0, 1.5, and 2 years. In the 2-year feeding study, no significant differences occurred in weight gain, food efficiency (body wt gain/100 g of diet), hemopoiesis, blood glucose, blood nonprotein nitrogen, serum calcium, urine albumin, urine sugar, and organ weights in treated rats as compared with controls. The histopathological evaluation of liver, kidney, spleen, heart, adrenals, thyroid, and gonads also revealed no differences. Results of the evaluations to determine whether $CaNa_2EDTA$ interfered with various aspects of mineral metabolism were also negative. There was no evidence of abnormal calcification in the tibias of rats sacrificed at 12 weeks, and at the end of the 2-year treatment, the ash content of the tibias of control rats was the same as that in rats fed the highest dose level. No effects were observed on two metalloenzymes: blood carbonic anhydrase, whose cofactor is zinc, and liver xanthine oxidase, which requires molybdenum and iron. The results on reproduction and lactation in four successive generations showed that $CaNa_2EDTA$ had no effect on fertility, gestation, viability, or lactation indices, and the 12-week growth responses for all dose levels were as good or better than those of control groups of the same sex and generation. This study was reviewed by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) and was used by them to calculate the ADI of $CaNa_2EDTA$ (JECFA, 1966, 1974).

Carcinogenicity

A rodent bioassay to determine the possible carcinogenicity of trisodium EDTA (Na_3EDTA) was reported by the National Cancer Institute (NCI, 1977). The test compound was administered for 103 weeks to Fischer 344 rats and B6C3F1 mice at dietary levels of 0.375 and 0.75%. Various tumors were observed in test and control groups of both species; however, they were not dose-related, and it was concluded that there was no statistical evidence of carcinogenicity for EDTA at the concentrations tested. Survival was satisfactory and there were no other signs of clinical toxicity.

Genotoxicity

EDTA has been tested for genotoxicity in assays measuring gene mutation and chromosomal effects. Dunkel *et al.* (1985) reported the results from a four-laboratory

study in which Na_3EDTA was tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 and in *Escherichia coli* WP2 *uvrA* both without metabolic activation and with activation, using liver S-9 preparations from Aroclor 1254-induced Fischer 344 rats, B6C3F1 mice, and Syrian hamsters. The number of revertants did not increase with any combination of bacterial tester strain or activation preparation. McGregor *et al.* (1988) reported that Na_3EDTA did not increase the mutant frequency in the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay in either the absence or presence of a metabolic activation system. In studies sponsored by the National Toxicology Program (unpublished data), Na_3EDTA was tested for its ability to induce chromosomal aberrations or sister chromatid exchanges in mammalian cells in culture. The results from these tests were also negative. Based on the above studies, EDTA is apparently not genotoxic.

Allergic Reactions

In an early human skin sensitization study, Raymond and Gross (1969) reported a 6% (3/50) positive response to $CaNa_2EDTA$, with 2 of the 3 positively responding individuals cross-reacting with ethylenediamine. They concluded: "In view of its widespread use and the absence of previously reported reactions, we feel it must be a weak sensitizing compound." Subsequently, Henck *et al.* (1985) evaluated the sensitizing potential of Na_3EDTA in guinea pigs and, based on their negative results, concluded that the potential for sodium salts of EDTA to produce a human skin sensitization response was extremely low.

Teratogenicity

There have been several studies in which the teratogenicity of EDTA was investigated (Swenerton and Hurley, 1971; Kimmel, 1977; Schardein *et al.*, 1981). Swenerton and Hurley (1971) reported that when pregnant female rats were fed diets containing 20 and 30 g Na_2EDTA/kg diet, gross congenital malformations were observed in the full-term young. However, when Na_2EDTA was fed simultaneously in a diet supplemented with zinc from the basal diet level of 100 to 1000 mg Zn/kg diet, no effects were observed. They suggested that the congenital effects may have resulted from zinc deficiency caused by the chelation of zinc by EDTA.

Kimmel (1977) administered Na_2EDTA to pregnant rats by different routes and at different dose levels. In rats fed 3% Na_2EDTA (954 mg Na_2EDTA/kg/day) in a semi-purified diet containing 50 mg Zn/kg diet, maternal body weight was severely reduced but no maternal deaths occurred; malformations were observed in 71% of the offspring. In dams given 750 mg Na_2EDTA/kg body wt by gavage twice daily (1500 mg Na_2EDTA/kg/day), the maternal death rate was 87.5%, whereas in rats given 625 mg Na_2EDTA/kg body wt by gavage twice daily (1250 mg Na_2EDTA/kg/day), the maternal death rate was 36% and malformations were observed in 20.5% of the offspring. Although a marked loss in maternal body weight and a death rate of 24% were observed in animals injected subcutaneously with 375 mg Na_2EDTA/kg body wt, the number of malformations in the offspring was not significant. Kimmel (1977) states that absorption of Na_2EDTA from the gut is normally quite low, but may have been greater in animals on dietary Na_2EDTA because of continual presentation of the compound,

or that the Na_2EDTA may have been altered by dietary components to form a more lipid-soluble complex.

The teratogenic capacity of EDTA and four of its salts, disodium, trisodium, calcium disodium, and tetrasodium EDTA, was reported by Schardein *et al.* (1981). Equimolar doses of the compounds, based on 1000 mg/kg body wt, were given by gastric intubation to pregnant rats receiving Purina Lab Chow during the period of organogenesis. No teratogenic effects were observed with any of the compounds, even at maternally toxic doses.

In the studies by Swenerton and Hurley (1971) and Kimmel (1977), Na_2EDTA induced teratogenic effects when the level of dietary zinc was relatively low. The discrepancy between the teratogenicity effects observed by Kimmel (1977) and Schardein *et al.* (1981) may be due to different levels of zinc in the diet, drinking water, or cage composition.

ACCEPTABLE DAILY INTAKE (ADI)

FAO/WHO has determined an ADI for EDTA based on the 1963 study by Oser *et al.* (1963). The ADI was calculated by using the highest dose tested (250 mg/kg body wt/day) as the no-observed-effect level, and by applying a safety factor of 100 to obtain a dose of 2.5 mg/kg body wt/day, which is equivalent to 150 mg/person/day for a 60-kg individual. $CaNa_2EDTA$ was used to calculate this dose.

In a comparison of the relative toxicities of Na_2EDTA and $CaNa_2EDTA$, JECFA (1974) concluded that the use of $CaNa_2EDTA$ is preferable to that of Na_2EDTA, since Na_2EDTA is more effective at sequestering calcium physiologically. However, JECFA stipulated that Na_2EDTA may be used when needed in foods, provided that it is used only at the level necessary to achieve the desired technical effect. It is also important to consider zinc nutriture in relationship to EDTA toxicity. Swenerton and Hurley (1971) found that high doses of Na_2EDTA were teratogenic in the presence of low dietary zinc but had no effect when dietary zinc content was elevated. This finding indicates that EDTA may bind zinc, inhibiting its absorption and, thus, reducing zinc status, which may be a factor in the teratogenicity of Na_2EDTA.

The findings of the chronic study on EDTA by Oser *et al.* (1963) were used to determine the ADI, and a subsequent bioassay has supported the findings of this study (NCI, 1977). Survival in the NCI study was satisfactory and no significant neoplastic or nonneoplastic responses were observed. Therefore, no scientific basis exists for changing the ADI for EDTA.

ESTIMATED DAILY INTAKE (EDI) FOR EDTA IN THE UNITED STATES

FDA has determined the EDI for EDTA on several occasions since 1969. In 1992, the agency updated the estimate of chronic exposure to EDTA by using available food intake information and Monte Carlo simulation methodology to calculate a 90th percentile exposure (Rubinstein, 1981). The model used in the simulation is based on the expression for the exposure to a substance in the diet

$$EDI_x = \sum_{f=1}^{f} C_{xf} \cdot I_f$$

where, for an individual, the exposure to a substance (x) is the sum of the exposures from the intake of food (I) for all foods (f) in that individual's diet treated with the substance. The contribution from each food (f) is the intake of that food multiplied by the concentration of the substance (C_x) in that food.

The Monte Carlo technique uses a distribution of values for a variable instead of a discrete value. The lognormal distributions of food intake were used for foods for which EDTA treatment is regulated, and triangular distributions (minimum, typical, and maximum) were used to describe the use levels. The food intake distributions were taken from the Market Research Corporation of America (MRCA) Information Services (Northbrook, IL) 5-Year Menu Census (1982–1987), and the 1987–1988 U.S. Department of Agriculture–Nationwide Food Consumption Survey (USDA–NFCS) portion sizes were used. The maximum use levels were taken from the appropriate FDA regulations. For use level distribution, typical values were estimated to be 80–90% of the maximum; minimum values were estimated to be 60–70% of the maximum. Values in Table 2 are the expected (mean) values resulting from application of the following assumptions: All foods that can be treated with EDTA contain EDTA, and the presence of EDTA in food does not affect a consumer's choices relating to that food. The food categories for which intake distributions are available from MRCA tend to be broader than those in the regulations. For example, the regulations allow EDTA use in processed dry pinto beans. MRCA intake data are available only for the category "dried vegetables." Likewise, canned black-eyed peas, canned cooked chick-peas, and canned kidney beans may be treated, but intake data are available only for the broad category "canned vegetables." In cases where data were not available for the specific foods treated, we used intake distributions for broader food categories that encompass the specific food. Additionally, the highest food EDTA use level for those categories was used. The effects of these assumptions on the estimate of exposure to EDTA are twofold. First, by assuming that all foods are treated with EDTA, the number of people theoretically exposed to EDTA is maximized. Second, the use of broad food categories results in an overstatement of the intake of EDTA. Therefore, this estimate must be seen as an upper bound, overstating both the intake and extent of exposure. The distribution of intakes obtained through the use of our model is likely to reflect EDTA intake for those who regularly consume products that happen to be treated with EDTA. The FDA has no information for estimating the size of this population.

One measure of the conservatism of our EDTA estimate can be obtained by comparing it to the amount of EDTA disappearing into the food supply. The total quantity of EDTA salt reported by weight in the NAS update for 1987 was 186,000 pounds (NAS, 1989). This amount yields a per capita estimate of exposure of 1.6 mg/person/day, or 1/10 our estimate.

Mean exposures to EDTA from individually regulated foods are outlined in Table 2. Because of the varied uses for EDTA, we calculated that all consumers are eaters of EDTA; therefore, eaters-only exposure is equivalent to the total-sample exposure. The results of the Monte Carlo simulation are summarized in Table 3. Mean overall exposure to EDTA has been estimated to be 15 mg/person/day. In the Monte Carlo simulation the 90th percentile exposure is 26 mg/person/day and the 50th is 13 mg/day. The difference in formula weight between $CaNa_2EDTA$ and Na_2EDTA was not taken into account; if it had been, the effect on overall exposure would be a difference on the order of 1 mg/person/day. This updated EDI is substantially below the ADI

TABLE 2

EXPOSURE TO EDTA FROM INDIVIDUALLY REGULATED FOODS[a]

Food type[b]	Use level[c] (ppm)	Food intake[d] (mean, g/person/day)	EDTA exposure[e] (mean, μg/person/day)
Pickled cabbage and cucumbers (relishes)	183	2.7	504
Canned carbonated soft drinks			
Cola	27	77.6	2121
Fruity	27	24.9	681
Other	27	10.1	277
Canned white potatoes	100	1.0	102
Salad dressings			
Standardized	65	4.6	299
Nonstandardized	65	2.2	146
Distilled alcoholic beverages	20	0.9	18
Canned crabmeat	255	0.2	48
Canned clams	320	0.0	15
Cooked, canned vegetables	292	21.4	6228
Extruded egg	180	0.0	5
Beer	20	29.7	593
Margarine	63	5.4	340
Potato salad	90	13.0	1170
Dry pinto beans	762	1.0	787
Spice extractives	52	0.0	0
Canned fruit pie filling	475	0.3	134
Sauces	68	1.7	114
Gefilte fish balls	45	1.5	68
Cooked sausage	33	8.7	288
RTE cereal (bananas)	280	0.2	50
Aqueous multivitamins	138	0.3	35
Frozen white potatoes	90	12.5	1125
Total			15,124[f]

[a] Zero result from rounding.

[b] Food categories are taken from the Market Research Corp. of America's 5-Year Menu Census Survey.

[c] The figures are mean values resulting from the use of a triangular probability distribution of use levels. Maximum levels are taken from the *Code of Federal Regulations;* most likely levels were taken to be 80–90% of the maximum, and minimum levels were taken to be 60–70% of the maximum. See text for details of methods used.

[d] Lognormal distributions taken from the MRCA 5-Year Menu Census were used. Mean food intakes are shown.

[e] Values shown are the mean exposures determined in the Monte Carlo analyses. These values are different from the product of mean use levels and food intake because of the skewness of the lognormal distributions of food intakes, or rounding. See text for details of methods used.

[f] Total exposure 15,124 μg/person/day is equivalent to 15 mg/person/day.

of 150 mg/person/day and suggests that additional uses or forms of EDTA, such as the NaFe salt in selected foods, are possible.

SUMMARY

FAO/WHO has determined an ADI of 2.5 mg/kg body wt/day or 150 mg/person/day, calculated as $CaNa_2EDTA$, with the stipulation that no excess of Na_2EDTA

TABLE 3

MONTE CARLO[a] RESULTS

@RISK: Simulation statistics for EDTA
Worksheet: EDTA
Expected/mean result = 15 mg/person/day
Maximum result = 322.5
Standard deviation = 9.7
Simulations executed = 1
Iterations = 30,000
Percentile probabilities (chance < = shown value)
50% 13 mg/person/day
90% 26 mg/person/day

[a] Monte Carlo method (Rubinstein, 1981).

(except that needed for technical effect) remain in foods. No chronic toxicity studies have been performed on EDTA since the ADI was determined; therefore, there is no scientific basis for changing the ADI for EDTA.

Based on current consumption data, the FDA has estimated that the current daily mean intake of EDTA is 15 mg/person/day. This more reliable EDTA daily intake estimate could support the inclusion of significant new uses of EDTA, such as NaFeEDTA for iron fortification.

NaFeEDTA provides a highly bioavailable form of iron for food fortification and promotes the absorption of intrinsic food iron in a meal with low iron bioavailability. Iron supplied in the form of NaFeEDTA may be two to three times more bioavailable than $FeSO_4$ or food iron in some diets (Viteri *et al.*, 1978), and iron bioavailability of NaFeEDTA is not generally affected by adverse storage conditions or by food preparations such as cooking. Data also indicate that a lower level of fortification iron could be used to prevent iron deficiency anemia in a population relying mainly on a diet high in vegetables, which contain inhibitors of iron absorption such as phytate and limited amounts of enhancers of iron absorption (ascorbic acid and meat). Because NaFeEDTA is absorbed well in foods containing dietary inhibitors of iron, smaller amounts may be used for iron fortification. In many countries the number of processed foods in the food supply is low and the EDI for EDTA is correspondingly lower than that for the United States. The introduction of NaFeEDTA into the food supply for iron fortification in those countries should not present a toxicological concern. However, if NaFeEDTA were used in countries where clinical manifestations of zinc deficiency exist or where calculated zinc intakes are marginal, the effects of EDTA consumption on zinc nutriture should be examined.

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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

WORLD HEALTH ORGANIZATION

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

WHO FOOD ADDITIVES SERIES: 44

Prepared by the Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

World Health Organization, Geneva, 2000
IPCS - International Programme on Chemical Safety

SODIUM IRON ETHYLENEDIAMINE TETRAACETIC ACID (EDTA)

First draft prepared by Dr J.B. Greig

Joint Food Safety & Standards Group, Department of Health, London, United Kingdom

1. EXPLANATION

The sodium iron salt of ethylenediamine tetraacetate (EDTA) was evaluated by the Committee at its forty-first meeting (Annex 1,

reference 107), which provisionally concluded that sodium iron EDTA that met the tentative specifications prepared at the meeting would not present a safety problem when used in supervised food fortification programmes in iron-deficient populations. The Committee requested the results of additional studies to assess the site of deposition of iron administered in this form and to assess the metabolic fate of sodium iron EDTA after long-term administration..The Committee emphasized that its evaluation applied only to the use of sodium iron EDTA as a dietary supplement to be used under supervision, and expressed its concern about the potential for over-fortification of food because of the enhanced bioavailability of iron in this form.

Several studies were submitted for consideration at the present meeting in response to the Committee's request.

2. BIOLOGICAL DATA

2.1 Toxicological studies

2.1.1 Acute toxicity

A summary report of the results of determinations of the LD_{50} of sodium iron EDTA in rats and mice was submitted (Sichuan Provincial Sanitary and Anti-epidemic Station, 1998).

2.1.2 Short-term studies of toxicity

Rats

The experiment described below was conducted according to the OECD principles of GLP. After a pilot experiment, groups of 40 male Sprague-Dawley Crl:CD(R) BR rats aged five to six weeks were fed diets containing iron in the form of sodium ferric EDTA or ferrous sulfate heptahydrate ($FeSO_4$) to provide iron at concentrations of 35, 70, or 140 mg/kg of diet. The mean daily intakes of iron at the low, intermediate, and high doses were 2.8, 5.7, and 12 mg/kg bw per day with $FeSO_4$ and 2.8, 5.7, and 11 mg/kg bw per day with sodium iron EDTA. The animals were fed the diet for 31 days, at which time 20 animals in each group were killed, while the remaining 20 were continued on the diet for a further 30 days. A group of 10 untreated male rats were killed at the start of the experiment to establish the baseline levels of the parameters that were to be measured. The toxicolo-gical end-points that were studied included clinical signs, body weights, organ weights, food consumption, food conversion efficiency, haematological and clinical chemical end-points, and the pathological appearancce of the liver, spleen, and any gross lesions. The distribution of iron was evaluated by determining iron and total iron-binding capacity in plasma and non-haem iron in the liver, spleen, and kidneys.

No deaths occurred during the experiment, and the clinical signs observed are commonly seen in laboratory rats. Occasional statistically significant changes in food consumption were not obviously related to treatment. Food conversion efficiency was variable but showed some small, statistically significant changes during the first four weeks. The iron content of the diets was within 10% of the intended value in 25 of the 26 batches prepared for both the pilot and main experiments, but the content of the 26th batch was 13% lower than the intended 70 mg/kg of iron.

Administration of either $FeSO_4$ or sodium iron EDTA resulted in increased non-haem iron concentrations in the liver, spleen, and kidney when compared with the baseline values after 31 or 61 days of feeding, and the changes were related to dose except in the spleen at 61 days. The non-haem iron concentra-tion in these organs resulting from administration of sodium iron EDTA was always either significantly lower or not significantly different from the concentration resulting from administration of $FeSO_4$. The plasma iron concentration was statistically significantly higher after 31 days of the low dose of sodium iron EDTA than after the intermediate dose, but no other significant difference was seen at 31 or 61 days. There were no statistically significant differences in the total iron-binding capacity of blood plasma after 32 days of feeding, and the statistically significant differences seen at 62 days were minor and unrelated to administered dose or the iron compound. After 31 days of feeding, there were no statistically significant differences in terminal body weight or absolute or relative organ weights in treated animals. At 61 days, the absolute and relative thymus weights of rats given the low dose of sodium iron EDTA were significantly higher than those at the low dose of $FeSO_4$ or at the other doses of sodium iron EDTA. No other significant changes in body weight or organ weight were observed.

The haematological examinations showed a statistically significant difference in the mean corpuscular haemoglobin concentration at 32 days only between rats given sodium iron EDTA and those given the low dose of $FeSO_4$, but the difference was less than 1.5% and no difference was seen between individual groups treated with sodium iron EDTA or at 62 days. At 32 days, animals given the high dose of sodium iron EDTA also had a statistically significantly increased eosinophil count, but it did not appear to be related to treatment or dose. No significant changes were found in any group at 62 days. Some inconsistent changes were seen in clinical chemical end-points in blood plasma collected on days 32 and 62. The activity of alkaline phosphatase was greater in all groups at day 32 than at day 62 and was greater in the $FeSO_4$-treated groups than in those given sodium iron EDTA; although statistically significant differences were seen, there was no evidence of a dose-response relationship and the effect had disappeared by 62 days. A statistically significant increase in total bilirubin occurred at 32 days in the group given the intermediate dose of $FeSO_4$, but no effect was seen at 62 days. Statistically significant differences in sodium ion concentration at 32 and 62 days and in chloride ion concentration at 32 days did not appear to be related to biologically significant changes. An apparently dose-related decrease in calcium ion concentration in both treated groups at 62 days was considered by the study authors to be associated with observed changes in albumin and hence total protein concentrations. Whereas liver damage might have been a cause of the decreased plasma albumin concentration, the absence of any other indicators of liver damage (such as liver-derived plasma enzyme activities and histological changes) led the study authors to conclude that the changes were of no toxicological significance.

Histopathological examination of sections of the liver and spleen stained with haematoxylin and eosin did not reveal any dose-or treatment-related changes. Such observations as were made were considered to be unremarkable and occurred either sporadically or with

a uniform distribution across all groups. At 32 days, the accumulation of iron did not result in a positive Prussian blue reaction in stained sections of the liver. A general increase was seen in the spleen in all groups, but the reaction tended to be confined to the red pulp and was classified as very slight to slight, although increased staining tended to be found in spleens from the groups fed the doses providing high concentrations of iron. At 62 days, the Prussian blue staining of the liver sections did not suggest a dose-or treatment-related effect.

The changes seen in the spleens of all animals generally suggested a dose-and time-related increase in staining, with no evidence of a difference between the groups fed $FeSO_4$ and sodium iron EDTA (Appel, 1999).

A summary report of a 90-day study in rats given sodium iron EDTA in the diet was submitted (Su *et al.*, 1999).

2.1.3 Genotoxicity

A summary report of the results of tests for genotoxicity *in vitro* and *in vivo* was submitted (Sichuan Provincial Sanitary and Anti-epidemic Station, 1998).

Table 1. Results of assays for the genotoxicity of sodium iron ethylenediami tetraacetic acid (EDTA)

End-point	Test object	Concentration	Result
Reverse mutation	S. typhimurium TA97a, TA98, TA100, TA102, TA1535, TA1537, TA1538	Not reported	Negative[a]
Gene mutation	Tk locus in mouse lymphoma L5178Y cells	0.03-325 mg/ml as Fe	Positive and cytotoxic with S9 negative without

From Dunkel et al. (1999); sodium iron EDTA of 98% purity was used.
S9, 9000 × g supernatant of rat liver

2.1.4 Developmental toxicity

A summary report of the results of a study of teratogenicity in rats of sodium iron EDTA was submitted (Sichuan Provincial Sanitary and Anti-epidemic Station, 1998).

2.1.5 Special study of nutrition

Rats

Eight groups of eight weanling male Sprague-Dawley rats weighing about 45 g were randomized to receive a zinc-sufficient diet containing zinc at 30 mg/kg of diet or a zinc-deficient diet containing zinc at 6.1 mg/kg of diet. Three of the groups in each of these two blocks were fed a diet containing sodium iron EDTA, while the diet of the other group contained $FeSO_4$. The iron content of all

diets was 50.1 mg/kg, but two groups fed sodium iron EDTA in each block received a further dose of 300 or 800 mg/kg diet. After receiving the diets for 18 days, the animals were transferred to metabolism cages for three days to allow study of the balance of calcium, copper, and zinc. Animals that did not eat normally during this period were removed from the analysis, and, on day 21, all animals were killed and the right femur removed for analysis of calcium and zinc.

The rats given the zinc-sufficient diets ate significantly more food, gained more weight, and had a higher femur zinc content than those given the zinc-deficient diets. Changing from $FeSO_4$ to sodium iron EDTA had no significant effect on the zinc-sufficient rats but resulted in a significant increase in food intake and body-weight gain. Increasing the EDTA content of the diet significantly but inconsistently reduced the femur zinc content of the zinc-sufficient rats, while in the zinc-deficient rats increasing the EDTA content reduced both food intake and body-weight gain with no significant change in femur calcium or zinc content.

Zinc absorption (intake minus faecal excretion) and retention (intake minus (faecal excretion plus urinary excretion)) were both greater in the rats given the zinc-deficient diet. Fortification with sodium iron EDTA increased both parameters and also urinary zinc excretion, although only the latter appeared to be related to the dose of EDTA. The dietary intake of zinc, the nature of the iron compound used to supplement the diet, and the concentration of EDTA had no marked effect on the absorption or excretion of either copper or calcium except for a small but significant dose-related effect of EDTA concentration on urinary calcium excretion (Hurrell et al., 1994).

2.1.6 Observations in humans

The effect of various ratios of disodium EDTA to $FeSO_4$ on the absorption of isotopically labelled $FeSO_4$ was studied in 127 women, some of whom had depleted iron stores and some of whom were also anaemic. At molar ratios of EDTA:Fe of 1-0.25, the absorption of iron from a meal with low iron bioavaila-bility was increased (MacPhail et al., 1994).

In a random, cross-over study, 10 healthy women aged 23-49 and weighing 40-70 kg received diets fortified with 10 mg Fe from either $FeSO_4$ or sodium iron EDTA for 14 days, starting after a period of menstruation and with an interval of about four weeks. Other than the addition of sugar, butter, or jam to match the caloric requirements of each subject, each received a basal diet, and all of the water that was drunk was ultra-pure. The first five days of each arm of the study were used for dietary adaptation, and then a six-day chemical balance study was carried out in the course of which stable isotope-labelled calcium and zinc were administered.

Significant enhancement of apparent zinc absorption was seen with sodium iron EDTA as compared with $FeSO_4$ (34 ± 17% (SD) and 21 ± 4.4%). Urinary zinc excretion was also significantly increased, but the difference in zinc retention was not significant. Absorption of isotopic calcium was not affected by the iron compound used, although a small but statistically significant increase in urinary excretion of isotopic calcium was confirmed in the chemical balance study. Overall, no significant negative effect of sodium iron EDTA on the uptake and

excretion of zinc was observed (Davidsson et al., 1994).

After an overnight fast, test meals containing 5 mg of Fe as either $FeSO_4$ or sodium iron EDTA and a radioisotopic ^{59}Mn tracer were administered to one man and nine women, one of whom was post-menopausal. The activity of ^{59}Mn excreted in urine was measured in seven consecutive 24-h samples, and whole-body retention of ^{59}Mn was measured on days 0, 1, 2, 3, 4, 5, 6, 10, 14, 17, 21, 27, and 28. On day 28, the second arm of the cross-over study was started with feeding of the other iron compound and a second tracer dose of ^{59}Mn in a similar protocol. No statistically significant difference in manganese retention or urinary excretion was seen with the two iron compounds (Davidsson et al., 1998).

A double-blind intervention study was carried out in Guatemala over 32 months to test the efficacy in controlling iron deficiency of the addition of sodium iron EDTA to sugar. The study was carried out on a community basis and had been planned to involve two highland and two lowland communities of populations ranging from 1144 to 1726, with one community in each area as controls. It had been planned to obtain an age-and sex-stratified sample of 318 persons from each community for detailed dietary, parasitological, haematological, and biochemical studies. The detection of a high prevalence of anaemia in one of the planned control communities, however, resulted in its conversion to a treatment community. Additionally, although the initial response rates in the communities were 54-78%, application of exclusion criteria resulted in failure to achieve the target basal numbers. There were additional losses of subjects during the study. The sugar supply of the treated communities, which was already supplemented with vitamin A, was further fortified with sodium iron EDTA at 1 g/kg sugar, equivalent to an Fe concentration of 130 mg/kg sugar. Dietary surveys during the study showed that the mean (± SD) individual sugar intake ranged from 35 ± 16 to 38 ± 24 g/day.

Biochemical measures of indicators of iron status--haemoglobin concentration, saturation of serum iron-binding capacity, free erythrocyte protoporphyin concentration, serum ferritin concentration, and iron store mass--were reported from the basal and final surveys. The changes observed indicated an improvement in iron status in the treated populations, with a reduction in iron-deficiency

erythropoiesis. The authors reported that forti-fication of sugar with sodium iron EDTA resulting in the absorption of iron at 0.95-3.1 mg/day had no adverse effects on people of each sex of various ages (Viteri et al., 1995).

3. COMMENTS

A study specifically designed to address the Committee's concerns involved feeding male rats diets containing iron in two forms, $FeSO_4$ and sodium iron EDTA, each at three doses, for 62 days. The dietary concentrations provided iron intakes of 2.8, 5.7, and 11 mg/kg bw per day (sodium iron EDTA) or 12 mg/kg bw per day ($FeSO_4$). Generally, there was a dose-related increase in the amount of non-haem iron stored in liver, spleen, and kidney, but smaller or equal amounts of iron were taken up from the sodium iron EDTA-containing diet in comparison with that containing $FeSO_4$. There was no evidence that the total iron-binding capacity of blood plasma was altered by treatment with sodium iron EDTA. The Committee therefore concluded that there

was no evidence that administra-tion of iron in the form of sodium iron EDTA would result in greater uptake of iron than from an equivalent dietary concentration of $FeSO_4$ once the nutritional requirement for iron is satisfied. There was no evidence of adverse effects at the highest daily intake of iron, 11 mg/kg bw, which is 55 times greater than the proposed daily human intake of 0.2 mg/kg bw in food fortification programmes.

Short-term studies in rats and humans have shown no adverse effects of dietary intake of sodium iron EDTA on the balance of other minerals such as calcium, copper, manganese, and zinc. The results of an intervention study in iron-deficient populations in Guatemala demonstrated the efficacy of a diet supplemented with sodium iron EDTA in reducing the prevalence of iron deficiency in humans.

4. EVALUATION

The Committee considered that the data submitted satisfied its concerns about the use of sodium iron EDTA in food fortification programmes. It was aware of the results of studies of the acute toxicity, mutagenicity, teratogenicity, and 90-day toxicity in rats of sodium iron EDTA. Full reports of these studies were not available, but the information was not considered necessary for evaluating the safety of this compound. The Committee also received an assessment of the potential intake of sodium iron EDTA by consumers in the United States that would result from fortification of foodstuffs. The Committee was of the view that this assessment was not relevant to any proposed use of sodium iron EDTA as a food fortifier in areas of iron deficiency.

The Committee concluded that sodium iron EDTA could be considered safe for use in supervised food fortification programmes, when public health officials had determined the need for iron supplementation of the diet of a population. Such programmes would provide daily iron intakes of approximately 0.2 mg/kg bw.

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See Also:

Toxicological Abbreviations

Evaluation Of The Toxicity Of Various Slug Pellet Formulations Including Multiguard®, Against The Larvae Of A Non-Target Ladybird Species, *Harmonia conformis* (Boisduval)(Coleoptera: Coccinellidae).

1. Aims

To evaluate the toxicity of Multiguard® to a non-target beneficial arthropod species and to compare this toxicity with another commercially available pellet used for control of mollusc pests. A blank pellet containing no active ingredient was used as a control.

2. Test Species

Ladybirds (Coleoptera: Coccinellidae) and their larvae are known to be important predators in both garden and agricultural ecosystems where they have utilised as biological control agents, regulating the numbers of pest species especially aphids (Wilson 1960). *Harmonia conformis* (Boisduval) is common Victoria, Australia where it can be found on a range of plant species, wherever there are aphids which are their major prey. *H. conformis* was selected as a test organism since it is an important benefical arthropod in areas where mollusc control agents are likely to be broadcast.

3. Methods

Collection and maintenance of test species

Harmonia conformis were collected from vegetation in the Melbourne area. The ladybirds used in experiments have been in culture for almost 2 years - 3rd instar larvae were selected from these cultures for use in the tests (the 3rd instar is the final stage before pupation - care was taken to select only smaller individuals which were unlikely to pupate during the experiments as prior to pupation individuals do not feed (Horne, pers. comm.)). Cultures were kept in aquaria in natural daylight and were maintained on an artificial diet similar to dog food, supplemented with fresh aphids. Prior to testing, larvae were acclimmated to laboratory conditions (25°C and with a 12:12h light:dark lighting regime) in clear plastic containers (265 x 195 x 100 mm) containing plant material such as stalks and flower heads to provide cover. Larvae were fed in the same way as the cultures.

Test Materials

1. Multiguard® pellets (90 g/kg active ingredient, iron EDTA complex)
2. Baysol® pellets (20 g/kg active ingredient, methiocarb)
3. "Blank" control pellets[1]

Experimental Protocol

- All experiments were performed in a constant temperature facility at 25°C and with a 12:12h light:dark lighting regime.

[1] Blank pellets were produced by hand using white bleached wheat flour. This was moistened with a little distilled water to form a thick paste. Pellets were shaped by pressing a syringe tip into the mixture. Pellets were then dried in an oven at 45°C for 48h.

- Dry weights of each pellet before and after exposure to the test animals were recorded in order to estimate the quantity of pellet consumed during exposure. Pellets were dried for 48h at 45°C to obtain dry weights.
- Larvae weights were recorded prior to exposure.
- Tests were performed in small plastic tubs (30mm deep and 60mm diameter) with lids. The tubs were inverted and a piece of filter paper (Whatman® No. 1)) was placed into the lid and moistened with 0.5 ml of distilled water. Small holes were made in the roof of each tub to allow adequate ventilation of the test arenas.
- One preweighed pellet was added to each container and was positioned on the filter paper such that moisture would be absorbed. 10 replicates were performed for each pellet treatment.
- One preweighed *H. conformis* was introduced to each test arena - after 24 and 48 h the test chambers were observed and the condition of the larvae was recorded. Larvae were deemed dead if there was no response to mechanical stimulation. If the larva was a) unable to locomote in a normal manner b) positioned on it's dorsal side (ie on it's back with legs upwards) and was unable to right itself c) showed uncoordinated movement of appendages in response to stimulation, then it was said to be paralysed.
- After 48 h the pellet was removed, dried in an oven for 48 h at 45°C and the change in weight/weight of pellet consumed was calculated.
- Larvae were not re-used in subsequent experiments after exposure.

4. Results and Discussion

Raw data collected can be found in the Appendices.

The mean (± S.E.) fresh weight of larvae used in the tests was 16.01 ± 1.33 mg.

Figure 1 shows the response of the test larvae to 24 and 48 h exposure to the two pellet treatments and a control. It is evident that in both pellet treatments and the control there was mortality of the larvae. Since no active ingredient (a.i.) was present in the control pellets it is probable that the 20 % mortality observed after 48 h was due to random factors and the sensitivity of the larvae to experimental manipulation. The same level of mortality (20 % after 48 h) was observed in the Multiguard® trial - this too may be due to random mortality. However, Baysol® appeared to be toxic to *N. gravis* - after 24 h exposure there was 60 % mortality and 30 % paralysis, suggesting that the larvae were poisoned from feeding on the pellets. After 48 h, 90 % were dead and 10 % were paralysed. It can be seen from the raw data in Appendix 1 that there was no recovery from the paralysed state i.e. if a ladybird larva was paralysed after 24 h it was dead or still paralysed after 48 h.

Figure 2 details the mean consumption of pellets during the 48 h exposure period, calculated from the difference in dry weight of the pellet before and after exposure. The estimated mean active ingredient (a.i.) intake was calculated from this as follows:

estimated active ingredient intake = change in dry weight of the pellet X the proportion of active ingredient in the pellet formulation

Multiguard® contains 90g of a.i. per kg of pellet
Baysol® contains 20g of a.i. per kg of pellet

fig. 2. mean pellet consumption and estimated mean active ingredient (a.i.) ingested by *N. gravis* after 48 h exposure to 2 pellet formulations and a control.

treatment	mean change in pellet weight (mg) (± S.E)	estimated mean a.i. consumption (mg) (± S.E)
control	3.6 ± 1.24	-
Multiguard®	10.14 ± 1.58	0.913 ± 0.141
Baysol®	2.9 ± 1.14	0.0058 ± 0.0022

It can be assumed that *H. conformis* was feeding on the pellets during the exposure since in all cases there was a reduction in the mean dry weight of the pellets after 48 h exposure. More active ingredient was consumed in the Multiguard® treatments than the Baysol® treatment (0.913 ± 0.141 mg and 0.0058 ± 0.0022 mg respectively) but the resulting mortality was much higher in Baysol® suggesting that methiocarb (the active ingredient in Baysol®), is very toxic to *H. conformis* larvae, whereas iron EDTA (the active ingredient in Multiguard®) may not be.

It should be noted that the raw data for the consumption of pellets (Appendix 2) shows that in some instances that there is a negative weight change during the trial with Baysol® pellets i.e. the pellets *increased* in weight. This suggests that somehow there was an increase in weight of the pellet. The raw data suggests that an amount of pellet was ingested in many of the cases where an apparent increase in dry mass was seen since the larvae in question often became paralysed or died. The increase in dry weight may be due to fungal growth within the pellet, effectively increasing the weight despite the fact that some of the pellet was consumed Using the change in dry weight as a measure of pellet consumption may not be an accurate method, especially for such small changes in weight. This would also lead to erroneous determination of the dose of active ingredient consumed possibly explaining why there is no apparent trend between the dose of active ingredient consumed and the response observed (see Appendix 3 for raw data of a.i. intake and response).

5. Conclusions

There was 20 % mortality observed after *H. conformis* was exposed for 48 h to Multiguard® and blank control pellets in laboratory trials. This was probably due to random mortalities since it was not expected to see mortality in the trials with control pellets containing no active ingredient. Therefore Iron EDTA complex - the active ingredient against molluscs in Multiguard® - is probably not particularly harmful to *H. conformis*. However, Baysol® pellets effected 90 % mortality and 10 % paralysis in the ladybird larvae after 48 h exposure. Even allowing for the 20 % random mortality observed in the control, the results suggest that the larvae were poisoned by methiocarb, the active ingredient in Baysol®. Larvae which were paralysed after consuming methiocarb showed no recovery with time, indeed of the 40 % paralysed after 24 h, all 40 % were dead after a further 24 h.

It was not possible to determine the dose of pellet required to effect mortality since there was no apparent trend between active ingredient intake and response (see appendix 3). Using dry weight as an estimate of pellet consumption may not be accurate for such small changes in weight, as a result any estimate of active ingredient intake would also be inaccurate.

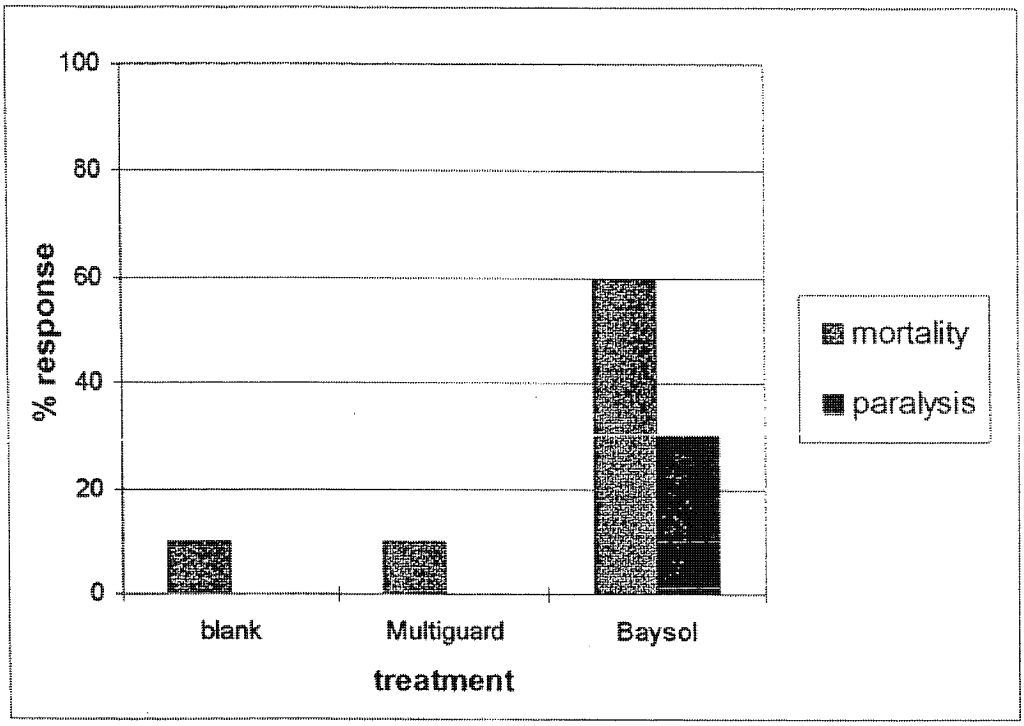
The results of laboratory tests suggest that the use Multiguard® pellets for the control of mollusc pests should have minimal if any adverse effects on *N. gravis*, a beneficial arthropod which is an important natural enemy of many pest species especially aphids (Wilson 1992). However, in identical tests Baysol® caused larval mortality at a level which could affect natural populations. Further field tests would be necessary to confirm the results of these laboratory trials.

6. References

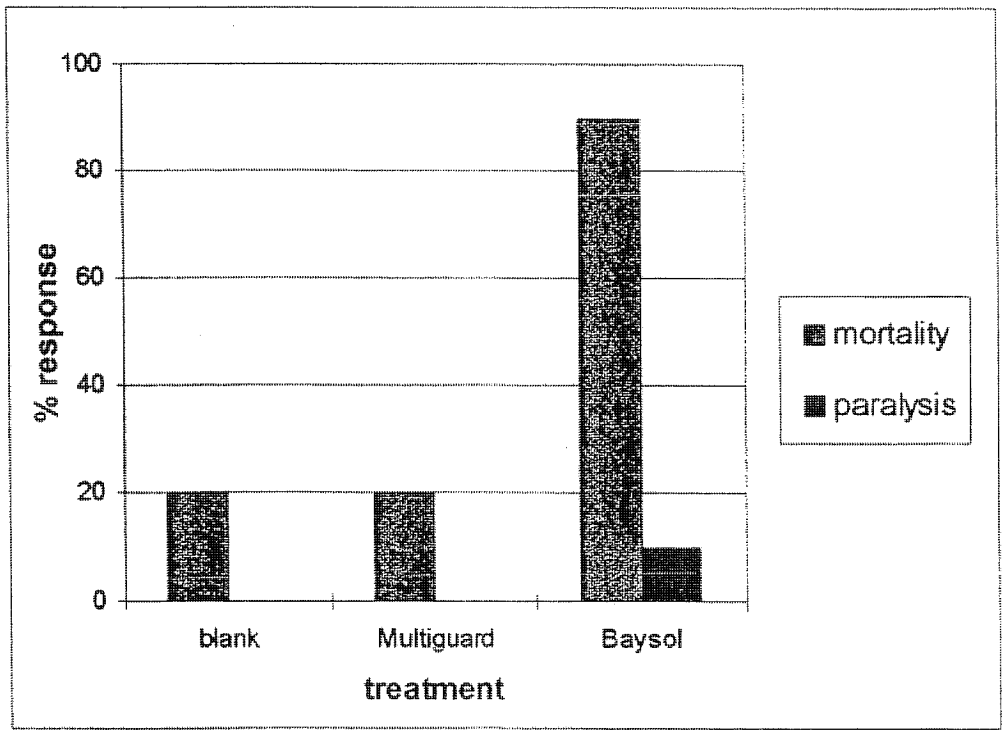
Wilson F (1960) A review of the biological control of insects and weeds in Australia and Australian New Guinea. CAB Publication, England. pp 6.

fig. 1. *Harmonia conformis* response to 24 and 48 h exposure to the two pellet treatments and a control

24 h exposure



48 h exposure



APPENDIX 1

Raw Data for Ladybird larvae tests

Individual larval response after 24 and 48 h exposure to two pellet treatments and a control (O - larva shows no adverse effects; X - larva dead; P - larva paralysed; PU - larva pupated during exposure (see Protocol section for explanation of categorisation))

replicate	response					
	blank		Multiguard		Baysol	
	24 h	48 h	24 h	48 h	24 h	48 h
1	O	O	O	O	X	X
2	X	X	O	O	X	X
3	O	O	O	O	P	X
4	O	O	X	X	P	P
5	O	O	O	O	X	X
6	O	O	PU	PU	X	X
7	O	PU	O	O	X	X
8	O	X	O	X	O	X
9	O	PU	O	O	P	X
10	O	O	O	O	X	X
% mortality	10	20	10	20	60	90
% paralysis	0	0	0	0	30	10
% pupation	0	20	10	10	0	0

APPENDIX 2

Pellet consumption after 48 h exposure

treatment	weight before mg	weight after mg	weight change mg	% weight change
blank	53.801	51.21	2.591	4.816
	133.301	132.29	1.011	0.758
	88.291	85.95	2.341	2.651
	113.811	109.11	4.701	4.131
	170.051	167.3	2.751	1.618
	113.121	104.09	9.031	7.983
	108.801	108.67	0.131	0.120
	110.611	109.97	0.641	0.580
	152.071	151.36	0.711	0.468
	120.161	108.29	11.871	9.879
mean	**116.402**	**112.824**	**3.578**	**3.300**
S.E.	**10.1**	**10.3**	**1.240**	**1.070**

treatment	weight before mg	weight after mg	weight change mg	% weight change
Multiguard	117.2	100.47	16.730	14.275
	104.88	95.91	8.970	8.553
	117.84	113.76	4.080	3.462
	110.67	109.74	0.930	0.840
	104.72	93.46	11.260	10.752
	79.01	67.83	11.180	14.150
	112.66	104.81	7.850	6.968
	93.12	82.55	10.570	11.351
	144.6	128.91	15.690	10.851
	123.65	109.5	14.150	11.444
mean	**110.835**	**100.694**	**10.141**	**9.265**
S.E.	**5.57**	**5.4**	**1.57**	**1.39**

treatment	weight before mg	weight after mg	weight change mg	% weight change
Baysol	73.377	64.62	8.757	11.934
	57.497	53.65	3.847	6.691
	71.667	65.24	6.427	8.968
	72.207	69.59	2.617	3.624
	56.077	55.88	0.197	0.351
	53.637	52.62	1.017	1.896
	57.187	53.75	3.437	6.010
	69.727	63.48	6.247	8.959
	66.067	67.74	-1.673	-2.532
	65.367	67.12	-1.753	-2.682
mean	**64.281**	**61.369**	**2.912**	**4.322**
S.E.	**2.38**	**2.1**	**1.12**	**1.59**

APPENDIX 3

Estimated intake of active ingredient (a. i.) and corresponding response of each larva after 24 and 48 h exposure to the test treatments.

The estimated intake of active ingredient is based on the change in dry weight of each pellet tested multiplied by the proportion of active ingredient in the pellet formulation (Multiguard® - 90g/kg; Baysol 20g/kg). The ratio of active ingredient to fresh body weight of each individual ladybird larva is given as an indication of the dose of a. i. with body size. The larval response to exposure is given after 24 and 48 h (O - larva shows no adverse effects; X - larva dead; P - larva paralysed; PU - larva pupated during exposure (see Protocol section for explanation of categorisation))

MULTIGUARD®

replicate	dry weight consumed (mg)	estimated a.i intake (mg)	body weight (mg)	ratio ai : body wt		response 24 h	response 48 h
1	16.73	1.50570	8.99	0.16749		O	O
2	8.97	0.80730	8.24	0.09797		O	O
3	4.08	0.36720	17.54	0.02094		O	O
4	0.93	0.08370	3.52	0.02378		X	X
5	11.26	1.01340	11.58	0.08751		O	O
6	11.18	1.00620	21.7	0.04637		PU	PU
7	7.85	0.70650	20.77	0.03402		O	O
8	10.57	0.95130	21.2	0.04487		O	X
9	15.69	1.41210	15.52	0.09099		O	O
10	14.15	1.27350	6.73	0.18923		O	O
mean	**10.14**	**0.913**	**13.58**	**0.08**	**% mortality**	10	20
s.e	**1.57**	**0.141**	**2.1**	**0.019**	**% paralysis**	0	0
					% pupation	10	10

BAYSOL®

replicate	dry weight consumed (mg)	estimated a.i intake (mg)	body weight (mg)	ratio ai : body wt		response 24 h	response 48 h
1	8.757	0.17514	9.26	0.01891		X	X
2	3.847	0.07694	6.09	0.01263		X	X
3	6.427	0.12854	20.96	0.00613		P	X
4	2.617	0.05234	29.76	0.00176		P	P
5	0.197	0.00394	25.81	0.00015		X	X
6	1.017	0.02034	23.34	0.00087		X	X
7	3.437	0.06874	19.39	0.00355		X	X
8	6.247	0.12494	19.02	0.00657		O	X
9	-1.673	-0.03346	11.99	-0.00279		P	X
10	-1.753	-0.03506	11.95	-0.00293		X	X
mean	**2.912**	**0.058**	**17.76**	**0.004**	**% mortality**	**60**	**90**
s.e	**1.12**	**0.022**	**2.43**	**0.002**	**% paralysis**	**30**	**10**

An Evaluation Of The Toxicity Of Various Slug Pellet Formulations, Including Multiguard®, Against A Non-Target Carabid Beetle Species, *Notonomus gravis* (Chaudoir).

1. Aims

To evaluate the toxicity of Multiguard® to a non-target beneficial arthropod species and to compare this toxicity with another commercially available pellet used for control of mollusc pests. A blank pellet containing no active ingredient was used as a control.

2. Test Species

Many carabids in a variety of ecosystems are known to be important predators in that they regulate the numbers of other invertebrates, including pest species (Luff 1987). *Notonomus gravis* (Chaudoir)(Coleoptera: Carabidae) inhabits the western grassland plains of Victoria, Australia and is the dominant carabid in this habitat - it is thought to have potential as a biological control agent (Horne 1992). *N. gravis* was selected as a test organism since it is an important benefical arthropod in areas where mollusc control agents are likely to be broadcast.

3. Methods

Collection and maintenance of test species

Notonomus gravis were collected using the pitfall trap technique in open grassland in North East Melbourne (Grid Ref. 37.45S 144.58E). Pitfall traps consisted of plastic tubs set flush with the ground, each 50mm deep and 110 mm diameter. Animals were maintained in the laboratory to provide individuals for experiments following the method of Horne (1992). Beetles were placed in clear plastic containers (265 x 195 x 100 mm) half-filled with damp peat moss and fed moistenend pellets of commercial dog food (LUV®). These carabids are predatory/scavengers and readily accept dog food.

Test Materials

1. Multiguard® pellets (90 g/kg active ingredient, iron EDTA complex)
2. Baysol® pellets (20 g/kg active ingredient, methiocarb)
3. "Blank" control pellets[1]*

Experimental Protocol

- All experiments were performed in a constant temperature facility at 25°C and with a 12:12h light:dark lighting regime.
- Dry weights of each pellet before and after exposure to the test animals were recorded in order to estimate the quantity of pellet consumed during exposure. Pellets were dried for 48h at 45°C to obtain dry weights.

[1] * Blank pellets were produced by hand using white bleached wheat flour. This was moistened with a little distilled water to form a thick paste. Pellets were shaped by pressing a syringe tip into the mixture and then expelling the resulting 'pellet' - these were then dried in an oven at 45°C for 48h.

- Beetle weights were recorded prior to exposure. Beetles were also sexed (males possess a thick brush of setae on the first tarsus of the foreleg, this is absent in females (Horne, pers. comm.)).
- Tests were performed in plastic tubs (50mm deep and 110mm diameter) with lids. The tubs were inverted and a filter paper (Whatman® No. 1)(110 mm diameter) was placed into the lid and moistened with 1ml of distilled water. Small holes were made in the roof of each tub to allow adequate ventilation of the test arenas.
- One preweighed pellet was added to each container and was positioned on the filter paper such that moisture would be absorbed. 10 replicates were performed for each pellet treatment.
- One preweighed male *N. gravis* was introduced to each test arena - after 24 and 48 h the test chambers were observed and the condition of the beetles was recorded. Beetles were deemed dead if there was no response to mechanical stimulation. If the beetle was a) unable to locomote in a normal manner b) positioned on it's dorsal side (ie on it's back with legs upwards) and was unable to right itself c) showed uncoordinated movement of appendages in response to stimulation, then it was said to be paralysed.
- After 48 h the pellet was removed, dried in an oven for 48 h at 45°C, and the change in weight (assumed to be the weight of pellet consumed by the beetle) was calculated.
- Beetles were not re-used in subsequent experiments after exposure.

4. Results and Discussion

Raw data can be found in the Appendices.

The mean (± S.E.) fresh weight of beetles used in the tests was 173.6 mg (± 6.16).

Figure 1 shows the response of the test carabids to 24 and 48 h exposure to the two pellet treatments and a control. It is evident that only Baysol® was toxic to *N. gravis* after 48 h exposure. After 24 h exposure to Baysol®, there was 20 % mortality and 30 % paralysis among the tested beetles. After 48 h, 40 % were dead and 30 % were paralysed. It can be seen from the raw data in Appendix 1 that there was no recovery from the paralysed state i.e. if a beetle was paralysed after 24 h it was dead or still paralysed after 48 h. In both Multiguard® and the control treatment there were no dead or paralysed beetles.

Figure 2 details the mean consumption of pellets during the 48 h exposure period, calculated from the difference in dry weight of the pellet before and after exposure. The estimated mean active ingredient (a.i.) intake was calculated from this as follows:

estimated active ingredient intake = change in dry weight of the pellet X the proportion of active ingredient in the pellet formulation

Multiguard® contains 90g of a.i. per kg of pellet
Baysol® contains 20g of a.i. per kg of pellet

In all cases there was a reduction in the mean dry weight of the pellets after 48 h exposure, therefore it can be assumed that *N. gravis* was feeding on the pellets

fig. 2. mean pellet consumption and estimated mean active ingredient (a.i.) ingested by *N. gravis* after 48 h exposure to 2 pellet formulations and a control.

treatment	mean change in pellet weight (mg) (± S.E)	estimated mean a.i. consumption (mg) (± S.E)
control	1.13 ± 0.418	-
Multiguard®	4.22 ± 0.551	0.38 ± 0.05
Baysol®	0.279 ± 0.331	0.006 ± 0.007

during the exposure (indeed mandible marks produced during beetle feeding could be seen on the pellets). There was no mortality or paralysis caused by feeding on Multiguard® pellets where a mean weight of 0.38 ± 0.05 mg of active ingredient was consumed. The mean weight of active ingredient in Baysol® (methiocarb) consumed (0006 mg ± 0.007) effected mortality in 40 % and paralysis in 30 % of the tested beetles.

It should be noted that the raw data for the consumption of pellets (Appendix 2) shows that in some instances that there is a negative weight change during the trial with Baysol® pellets i.e. the pellets *increased* in weight. The raw data suggests that an amount of pellet was ingested in many of the cases where an apparent increase in dry mass was seen since the slater in question often became paralysed or died. The increase in dry weight may be due to fungal growth within the pellet, effectively increasing the weight despite the fact that some of the pellet was consumed. Using the change in dry weight as a measure of pellet consumption may not be an accurate method, especially for such small changes in weight. This would also lead to erroneous determination of the dose of active ingredient consumed possibly explaining why there is no apparent trend between the dose of active ingredient consumed and the response observed (see Appendix 3 for raw data of a.i. intake and response).

5. Conclusions

There were no adverse affects to *N. gravis* after 48 h exposure to Multiguard® and blank control pellets in laboratory trials. However, Baysol® pellets effected mortality and paralysis after 24 h exposure, and after 48 h even more beetles were affected. The Iron EDTA complex - the active ingredient against molluscs in Multiguard® - is not harmful to *N. gravis*. However, methiocarb, the active ingredient in Baysol®, is toxic to *N. gravis* and in laboratory tests it has induced paralysis or death after ingestion in 70% of the beetles tested. Beetles which were paralysed after consuming methiocarb showed no recovery with time

It was not possible to determine the dose of pellet required to effect mortality since there was no apparent trend between active ingredient intake and response (see appendix 3). Using dry weight as an estimate of pellet consumption may not be accurate for such small changes in weight, as a result any estimate of active ingredient intake would also be inaccurate.

The results of laboratory tests suggest that the use Multiguard® pellets for the control of mollusc pests should have no adverse effects on *N. gravis*, a beneficial arthropod which is an important natural enemy of many pest species (Horne 1992). However, in identical tests Baysol® caused beetle mortality at a level which could affect natural beetle populations. Further field tests would be necessary to confirm the results of these laboratory trials.

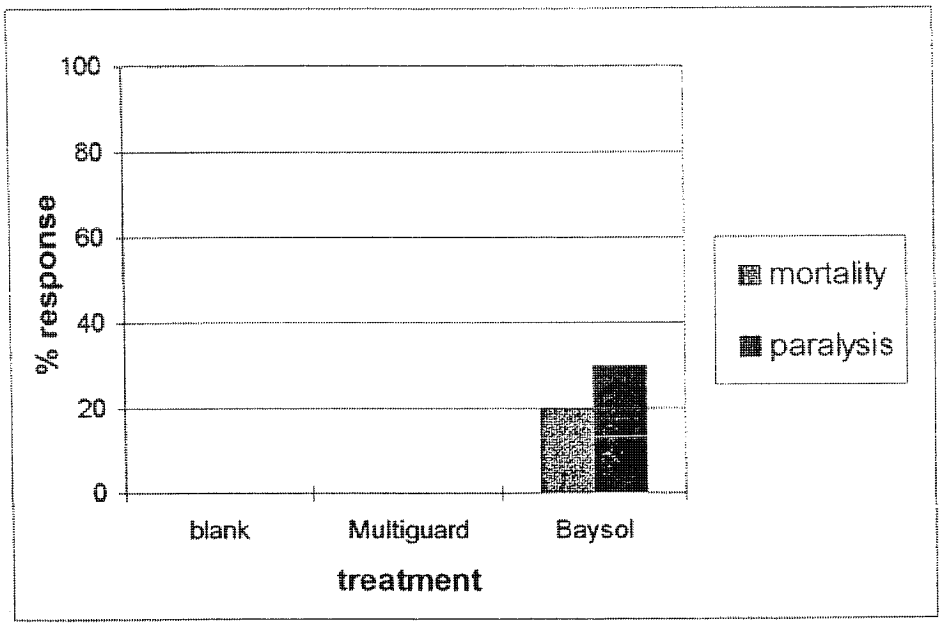
6. References

Horne, P. A. (1992) Comparative Life Histories of Two Species of *Notonomus* (Coleoptera: Carabidae) in Victoria. *Australian Journal of Zoology* **40**, 163-171.

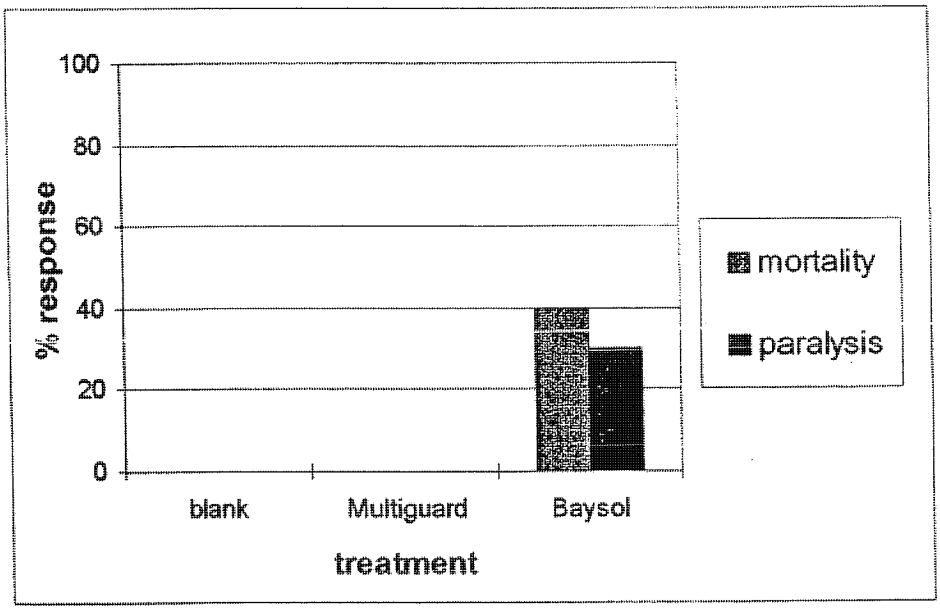
Luff, M. L. (1987) Biology of Polyphagous ground beetles in agriculture. *Agricultural Zoology Reviews* **2**, 237-278.

fig. 1. *N. gravis* response to 24 and 48 h exposure to the two pellet treatments and a control

24 h exposure



48 h exposure



APPENDIX 1

Raw Data for Carabid tests

Individual beetle response after 24 and 48 h exposure to two pellet treatments and a control (O - beetle shows no adverse effects; X - beetle dead; P - beetle paralysed (see Protocol section for explanation of categorisation))

replicate	response					
	blank		Multiguard		Baysol	
	24 h	48 h	24 h	48 h	24 h	48 h
1	O	O	O	O	O	P
2	O	O	O	O	P	X
3	O	O	O	O	X	X
4	O	O	O	O	X	X
5	O	O	O	O	O	O
6	O	O	O	O	O	P
7	O	O	O	O	P	X
8	O	O	O	O	P	P
9	O	O	O	O	O	O
10	O	O	O	O	O	O
% mortality	**0**	**0**	**0**	**0**	**20**	**40**
% paralysis	**0**	**0**	**0**	**0**	**30**	**30**

APPENDIX 2

Pellet consumption after 48 h exposure

treatment	weight before mg	weight after mg	weight change mg	% weight change
blank	160.841	157.050	3.791	2.357
	123.971	121.540	2.431	1.961
	128.251	127.780	0.471	0.367
	125.181	124.020	1.161	0.927
	106.861	105.520	1.341	1.255
	147.221	145.910	1.311	0.890
	138.631	138.180	0.451	0.325
	158.281	157.260	1.021	0.645
	131.111	130.550	0.561	0.428
	142.681	143.920	-1.239	-0.868
mean	**136.303**	**135.173**	**1.130**	**0.829**
S.E.	5.25	5.2	0.418	0.286

treatment	weight before mg	weight after mg	weight change mg	% weight change
Multiguard	93.100	91.090	2.010	2.159
	122.130	114.580	7.550	6.182
	102.810	99.830	2.980	2.899
	102.930	98.970	3.960	3.847
	128.480	124.080	4.400	3.425
	125.990	120.940	5.050	4.008
	101.120	97.970	3.150	3.115
	80.150	77.150	3.000	3.743
	119.720	113.080	6.640	5.546
	123.480	120.020	3.460	2.802
mean	**109.991**	**105.771**	**4.220**	**3.773**
S.E.	**5.14**	**4.8**	**0.551**	**0.393**

treatment	weight before mg	weight after mg	weight change mg	% weight change
Baysol	58.677	57.490	1.18700	2.023
	39.397	39.950	-0.55300	-1.404
	42.807	43.490	-0.68300	-1.596
	45.487	45.650	-0.16300	-0.358
	56.087	56.120	-0.03300	-0.059
	57.597	56.250	1.34700	2.339
	72.297	70.910	1.38700	1.918
	30.117	30.890	-0.77300	-2.567
	80.017	78.150	1.86700	2.333
	39.167	39.960	-0.79300	-2.025
mean	**52.165**	**51.886**	**0.279**	**0.061**
S.E.	**4.96**	**4.66**	**0.331**	**0.615**

APPENDIX 3

Estimated intake of active ingredient (a. i.) and corresponding response of each beetle after 24 and 48 h exposure to the test treatments.

The estimated intake of active ingredient is based on the change in dry weight of each pellet tested multiplied by the proportion of active ingredient in the pellet formulation (Multiguard® - 90g/kg; Baysol 20g/kg). The ratio of active ingredient to fresh body weight of each individual beetle is given as an indication of the dose of a. i. with body size. The beetle response to exposure is given after 24 and 48 h (O - beetle shows no adverse effects; X - beetle dead; P - beetle paralysed (see Protocol section for explanation of categorisation))

MULTIGUARD®

replicate	dry weight consumed (mg)	estimated a.i intake (mg)	body weight (mg)	ratio ai : body wt		response 24 h	response 48 h
1	2.010	0.18090	261.000	0.00069		O	O
2	7.550	0.67950	190.000	0.00358		O	O
3	2.980	0.26820	139.000	0.00193		O	O
4	3.960	0.35640	181.000	0.00197		O	O
5	4.400	0.39600	192.000	0.00206		O	O
6	5.050	0.45450	219.000	0.00208		O	O
7	3.150	0.28350	142.000	0.00200		O	O
8	3.000	0.27000	182.000	0.00148		O	O
9	6.640	0.59760	147.000	0.00407		O	O
10	3.460	0.31140	157.000	0.00198		O	O
mean	**4.22**	**0.38**	**181**	**0.002**	**% mortality**	0	0
s.e	**0.551**	**0.05**	**12**	**3E-04**	**% paralysis**	0	0

BAYSOL®

replicate	dry weight consumed (mg)	estimated a.i intake (mg)	body weight (mg)	ratio ai : body wt		response 24 h	response 48 h
1	1.187	0.0237	163.000	0.0001		O	P
2	-0.553	-0.0111	154.000	-0.0001		P	X
3	-0.683	-0.0137	186.000	-0.0001		X	X
4	-0.163	-0.0033	184.000	0.0000		X	X
5	-0.033	-0.0007	170.000	0.0000		O	O
6	1.347	0.0269	203.000	0.0001		O	P
7	1.387	0.0277	170.000	0.0002		P	X
8	-0.773	-0.0155	134.000	-0.0001		P	P
9	1.867	0.0373	136.000	0.0003		O	O
10	-0.793	-0.0159	156.000	-0.0001		O	O
mean	**0.279**	**0.006**	**165.6**	**3E-05**	**% mortality**	**20**	**40**
s.e	**0.331**	**0.007**	**6.91**	**4E-05**	**% paralysis**	**30**	**30**

Evaluation of the secondary toxicity of snails previously poisoned by pellet formulations including Multiguard® to a non-target carabid beetle species, *Notonomus gravis* (Chaudoir).

1. Aims

To evaluate whether there was any secondary toxicity of various slug pellet formulations including Multiguard® to a non-target beneficial arthropod species - in order to do this, snails which had previously been exposed to the test pellets and which displayed symptoms of poisoning (paralysis or death) were offered to carabid beetles in laboratory trials. The carabids were monitored for 48 h to determine whether there was any poisoning caused by ingestion of snail tissues which may have been contaminated by the active ingredients from the various pellet formulations.

2. Test Species

The Brown Garden Snail *Helix aspersa* Müller, of European origin, has been introduced into almost every country affected by European settlement (Dees 1970). It has become a pest of gardens and citrus groves throughout temperate regions including south east Australia (Young 1996).

Many carabids in a variety of ecosystems are known to be important predators in that they regulate the numbers of other invertebrates, including pest species (Luff 1987). *Notonomus gravis* (Chaudoir)(Coleoptera: Carabidae) inhabits the western grassland plains of Victoria, Australia and is the dominant carabid in this habitat - it is thought to have potential as a biological control agent (Horne 1992). *N. gravis* was selected as a test organism since it is an important beneficial arthropod in areas where mollusc control agents are likely to be broadcast. Carabids have been reported to be important predators in the control of mollusc populations such that the preservation of these species would beneficial for the biological control of snail and slug pests - it is possible that this species may play such a role in Victoria, although there is no literature to confirm this at present.

3. Methods

Collection and maintenance of test species

Adult *Helix aspersa* (shell height > 19 mm) were collected in the Melbourne area and were maintained in the laboratory in large plastic storage boxes (600 x 300 x 300 mm) lined with damp peat moss. Snails were fed a combination of sliced carrot, lettuce and $CaCO_3$ was provided following the method of Schuytema *et al.* (1994). Snails were starved for 24 h prior to exposure in order that each individual was in a similar physiological state.

Notonomus gravis were collected using the pitfall trap technique in open grassland in North East Melbourne. Pitfall traps consisted of plastic tubs set flush with the ground, each 50mm deep and 110mm diameter. Animals were maintained in the laboratory to provide individuals for experiments following the method of Horne (1992). Beetles were placed in clear plastic containers (265 x 195 x 100 mm) half-filled with damp peat moss and fed moistened pellets of commercial dog food (LUV®). These carabids are predatory/scavengers and readily accept dog food.

Test Materials

1. Multiguard® pellets (90 g/kg active ingredient -Iron EDTA complex)
2. Baysol pellets (20 g/kg active ingredient - Methiocarb)
3. "Blank" control pellets[1]

Experimental Protocol a) Snail exposures

- All experiments were performed in a constant temperature facility at 25°C and with a 12:12h light:dark lighting regime.
- Dry weights of each pellet before and after exposure to the test animals were recorded in order to estimate the quantity of pellet consumed during exposure. Pellets were dried for 48h at 45°C to obtain dry weights.
- Snail weights and shell heights were recorded prior to exposure.
- Tests were performed in plastic tubs (50mm deep and 110mm diameter) with lids. The tubs were inverted and a filter paper (Whatman® No. 1)(110 mm diameter) was placed into the lid and moistened with 1ml of distilled water. Small holes were made in the roof of each tub to allow adequate ventilation of the test arenas.
- One preweighed pellet was added to each container and was positioned on the filter paper such that moisture would be absorbed. 6-10 replicates were performed for each pellet treatment.
- One preweighed snail was introduced to each test arena and after 24 and 48 h the test chambers were observed and the condition of the snails was recorded. Snails were deemed dead if there was no response to mechanical stimulation. If the snail responded to stimulation but was unable to crawl in a co-ordinated manner then it was said to be paralysed.
- After 48 h the pellet was removed, dried in an oven for 48 h at 45°C, and the change in weight (assumed to be the weight of pellet consumed by the snail) was calculated.

b) *beetle exposures*

- Beetle weights were recorded prior to exposure. Beetles were also sexed (males possess a thick brush of setae on the first tarsus of the foreleg, this is absent in females (Horne, pers. comm.)).
- Tests were performed in plastic tubs (50mm deep and 110mm diameter) with lids. The tubs were inverted and a filter paper (Whatman® No. 1)(110 mm diameter) was placed into the lid and moistened with 1ml of distilled water. Small holes were made in the roof of each tub to allow adequate ventilation of the test arenas.
- One snail which had previously been exposed to the pellet treatments was added to each container. Care was taken that no pellet debris, which the beetle could potentially consume, was on the snail before introduction. 6-10 replicates were performed for each pellet treatment.
- One preweighed male *N. gravis* was introduced to each test arena - after 24 and 48 h the test chambers were observed and the condition of the beetles was recorded. Beetles were deemed dead if there was no response to mechanical stimulation. If the beetle was a) unable to locomote in a normal manner b) positioned on it's dorsal side (i.e. on it's back with legs upwards) and was unable

[1] Blank pellets were produced by hand using white bleached wheat flour. This was moistened with a little distilled water to form a thick paste. Pellets were shaped by pressing a syringe tip into the mixture. Pellets were then dried in an oven at 45°C for 48h.

to right itself c) showed uncoordinated movement of appendages in response to stimulation, then it was said to be paralysed.

4. Results and Discussion

Raw data can be found in the Appendices.

The mean (± S.E.) fresh weight and shell height of snails used in the tests was 5.186 □ 0.424 g and 25.05 ± 0.806 mm respectively. The mean (± S.E.) fresh weight of beetles used in the tests was 0.173 mg ± 0.006 g.

Snails

Figure 1 shows the response of the snails after 24 and 48 h exposure to the two pellet treatments and a control. There was no adverse response to the blank pellets. As expected there was significant mortality/paralysis in the snails exposed to both Multiguard® and Baysol®, the former seemed to effect mortality more rapidly (80% mortality, 0% paralysis after 48 h) than the latter (10 % mortality and 60 % paralysis after 48 h), the induced paralysis lasting longer in the Baysol® treatment.

Figure 2 details the mean consumption of pellets during the 48 h exposure period, calculated from the difference in dry weight of the pellet before and after exposure. The estimated mean active ingredient (a.i.) intake was calculated from this as follows:

estimated active ingredient intake = change in dry weight of the pellet X the proportion of active ingredient in the pellet formulation

Multiguard® contains 90g of a.i. per kg of pellet
Baysol® contains 20g of a.i. per kg of pellet

In all cases there was a reduction in the mean dry weight of the pellets after 48 h exposure. In some cases the entire pellet was consumed (Appendix 2 for raw data).

fig. 2. mean pellet consumption and estimated mean active ingredient (a.i.) ingested by *N. gravis* after 48 h exposure to 2 pellet formulations and a control.

treatment	mean change in pellet weight (mg) (± S.E)	estimated mean a.i. consumption (mg) (± S.E)
control	65.07 ± 21	-
Multiguard®	90.58 ± 17.0	8.97 ± 1.53
Baysol®	21.522 ± 6.4	0.4304 ± 0.128

More active ingredient was consumed in the case of Multiguard® (8.97 ± 1.53 mg) than Baysol® (0.4304 ± 0.128 mg), this may explain why snails were observed to die more rapidly (i.e. more were dead after 48 h) in the Multiguard® trial. Both the Iron

EDTA complex (the active ingredient in Multiguard®) and methiocarb (the active ingredient in Baysol®) were effective against *H. aspersa* in these trials.

It should be noted that the raw data for the consumption of pellets (Appendix 2) shows that in some instances that there is a negative weight change during the trial i.e. the pellets *increased* in weight. This may be due to mucous from the snails contaminating the pellets. In none of these cases were then snails poisoned indicating that they had not fed on the pellets.

Beetles

Figure 2 shows the response of the beetles after 24 and 48 h exposure to the two pellet treatments and a control. After introduction the beetles were observed actively feeding on poisoned and control snails whether they were dead, paralysed or had not been affected by the pellet treatments. There was no adverse response to the snails exposed to the blank pellets or to Multiguard®. However, after 24 h 10 % of beetles were dead in Baysol® treatments - after 48 h there was 20 % mortality and 10 % were paralysed. It would appear that there was a degree of secondary toxicity, after 48 h 30 % of the beetles had been poisoned by feeding on the tissues of snails which had previously consumed methiocarb (the active ingredient in Baysol®). Further experiments would need to be performed to confirm that this was indeed the case.

5. Conclusions

Both Multiguard® and Baysol® pellets effected mortality and paralysis in 80 and 70 %, respectively, of the tested snails, *H. aspersa*. However, there appeared to be no secondary toxicity to *N. gravis* when exposed to snails previously poisoned with Multiguard®. This may be expected as there was no mortality when *N. gravis* was exposed directly to Multiguard® pellets (see previous report). There was some secondary toxicity to beetles offered snails which had previously been exposed to Baysol® pellets. Methiocarb, the active ingredient in Baysol® pellets, had previously been shown to effect mortality in *N. gravis* when exposed to pellets directly - it is evident that methiocarb may have been present in sufficient concentrations in the snail tissues to poison the beetles. Further tests need to be performed in order to verify this.

The results of laboratory tests suggest that the use Multiguard® pellets for the control of mollusc pests should have no adverse effects on *N. gravis*, either by direct toxicity or indirectly by feeding on poisoned snails. However, in identical tests, snails previously poisoned by Baysol® caused beetle mortality at a level which could affect natural beetle populations. Further field tests would be necessary to confirm the results of these laboratory trials.

6. References

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Luff, M. L. (1987) Biology of Polyphagous ground beetles in agriculture. *Agricultural Zoology Reviews* **2**, 237-278.

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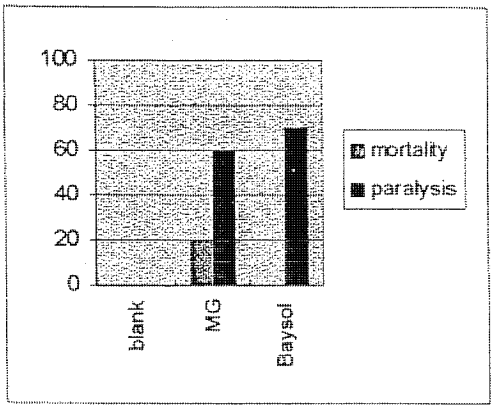
Young C (1996) Metal Chelates as stomach poison molluscicides for introduced pests, *Helix aspersa*, *Theba pisana*, *Cernuella virgata* and *Deroceras reticulatum* in Australia. In: *Slug and Snail Pests in Agriculture*. Henderson I F (ed.). BCPC Symposium Proceedings No. 66 pp. 237 - 243.

fig. 1. *H. aspersa* response to 24 and 48 h exposure to the two pellet treatments and a control (MG represents Multiguard® treatment)

24 h exposure



48 h exposure

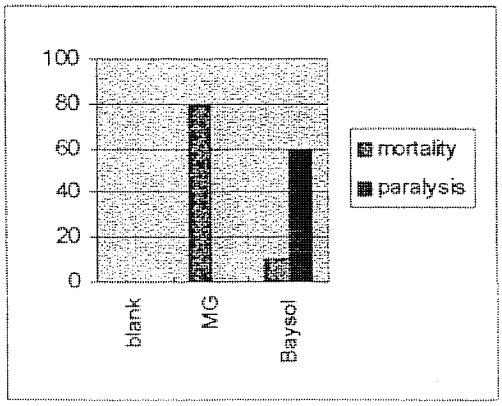
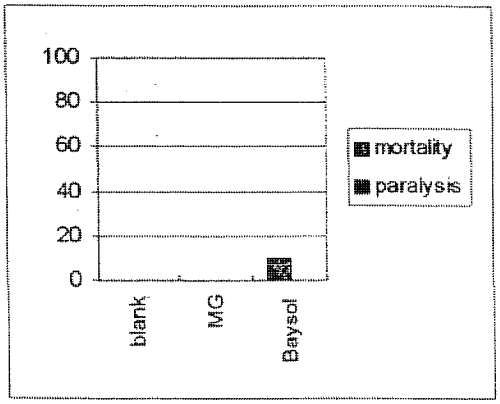
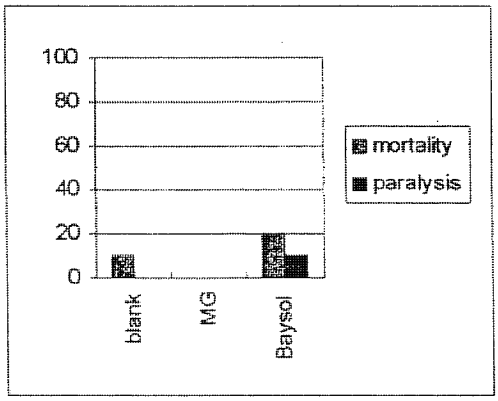


fig. 2. *N. gravis* response to 24 and 48 h exposure to snails previously exposed to the two pellet treatments and a control (MG represents Multiguard® treatment)

24 h exposure



48 h exposure



APPENDIX 1

Raw Data for Secondary Toxicity tests

Individual snail response after 24 and 48 h exposure to two pellet treatments and a control (O - snail shows no adverse effects; X - snail dead; P - snail paralysed (see Protocol section for explanation of categorisation))

replicate	response					
	blank		Multiguard		Baysol	
	24 h	48 h	24 h	48 h	24 h	48 h
1	0	0	P	X	0	0
2	0	0	0	0	P	P
3	0	0	0	0	P	P
4	0	0	P	X	0	0
5	0	0	X	X	P	P
6	0	0	X	X	P	P
7			P	X	P	P
8			P	X	P	P
9			P	X	P	X
10			P	X	P	P
% mortality	**0**	0	20	80	0	10
% paralysis	**0**	0	60	0	80	70

Individual beetle response after 24 and 48 h exposure to snails previously exosed to two pellet treatments and a control (O - snail shows no adverse effects; X - snail dead; P - snail paralysed (see Protocol section for explanation of categorisation))

replicate	response					
	blank		Multiguard		Baysol	
	24 h	48 h	24 h	48 h	24 h	48 h
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	X
4	0	0	0	0	0	0
5	0	0	0	0	X	X
6	0	0	0	0	0	0
7			0	0	0	0
8			0	0	0	0
9			0	0	0	P
10			0	0	0	0
% mortality	**0**	0	0	0	10	20
% paralysis	**0**	0	0	0	0	10

APPENDIX 2

Pellet consumption after 48 h exposure

treatment	weight before mg	weight after mg	weight change mg	% weight change
blank	96.910	0.000	96.9100	100.0000
	102.000	0.000	102.0000	100.0000
	108.200	0.000	108.2000	100.0000
	84.200	0.000	84.2000	100.0000
	102.910	103.400	-0.4900	-0.4761
	74.800	75.210	-0.4100	-0.5481
mean	94.8367	29.7683	65.0683	66.4960
S.E.	5.21	19.2	21	21.2

treatment	weight before mg	weight after mg	weight change mg	% weight change
Multiguard	131.030	0.000	131.030	100.000
	129.600	71.300	58.300	44.985
	133.360	2.490	130.870	98.133
	110.660	99.350	11.310	10.220
	128.820	69.650	59.170	45.932
	203.140	156.010	47.130	23.201
	181.620	20.180	161.440	88.889
	140.920	9.070	131.850	93.564
	177.370	40.830	136.540	76.980
	134.290	5.450	128.840	95.942
mean	133.710	43.121	90.589	61.622
S.E.	8.96	19	17	11.4

treatment	weight before mg	weight after mg	weight change mg	% weight change
Baysol	44.660	44.790	-0.130	-0.291
	59.760	0.000	59.760	100.000
	68.570	49.710	18.860	27.505
	56.040	56.680	-0.640	-1.142
	54.310	20.690	33.620	61.904
	62.950	7.720	55.230	87.736
	85.220	73.070	12.150	14.257
	54.710	44.990	9.720	17.766
	54.710	39.910	14.800	27.052
	65.140	52.830	12.310	18.898
mean	55.097	39.039	21.568	35.368
S.E.	3.17	6.69	6.4	10.7

Evaluation Of The Toxicity Of Various Slug Pellet Formulations, Including Multiguard®, Against A Non-Target Beetle Species, *Dicranolaius bellulus* (Guerin-Meneville)(Coleoptera: Melyridae).

1. Aims

To evaluate the toxicity of Multiguard® to a non-target arthropod species and to compare this toxicity with another commercially available pellet used for control of mollusc pests. A blank pellet containing no active ingredient was used as a control.

2. Test Species

Melyrid beetles are thought to be important predators in that they regulate the numbers of other invertebrates, including pest species (Horne pers. comm). *Dicranolaius bellulus* (Guerin-Meneville)(Coleoptera: Melyridae) inhabits the western grassland plains of Victoria, Australia and is often found in agricultural ecosystems where it is thought to have potential as a biological control agent (Pyke and Brown 1996). *D. bellulus* was selected as a test organism since it is an important beneficial arthropod in areas where mollusc control agents are likely to be broadcast.

3. Methods

Collection and maintenance of test species

Dicranolaius bellulus were collected in a potato crop and a carrot crop in Swan Hill, Victoria, Australia (Grid Ref. 35.23S 143.37E), which they invaded from the surrounding bush. Animals were maintained in the laboratory at 25°C with a 12:12h light:dark lighting regime. Beetles were placed in clear plastic containers (265 x 195 x 100 mm) containing plant material such as stalks and flower heads to provide cover. Beetles were fed moistened pellets of commercial dog food (LUV®) supplemented with fresh insects such as aphids and caterpillars (genus *Heliothus*).

Test Materials

1. Multiguard® pellets (90 g/kg active ingredient, iron EDTA complex)
2. Baysol® pellets (20 g/kg active ingredient, methiocarb)
3. "Blank" control pellets[1]

Experimental Protocol

- All experiments were performed in a constant temperature facility at 25°C and with a 12:12h light:dark lighting regime.
- Dry weights of each pellet before and after exposure to the test animals were recorded in order to estimate the quantity of pellet consumed during exposure. Pellets were dried for 48h at 45°C to obtain dry weights.
- Beetle weights were recorded prior to exposure.
- Tests were performed in plastic tubs (50mm deep and 110mm diameter) with lids. The tubs were inverted and a filter paper (Whatman® No. 1)(110 mm

[1] Blank pellets were produced by hand using white bleached wheat flour. This was moistened with a little distilled water to form a thick paste. Pellets were shaped by pressing a syringe tip into the mixture. Pellets were then dried in an oven at 45°C for 48h.

diameter) was placed into the lid and moistened with 1ml of distilled water. Small holes were made in the roof of each tub to allow adequate ventilation of the test arenas.

- One preweighed pellet was added to each container and was positioned on the filter paper such that moisture would be absorbed. 10 replicates were performed for each pellet treatment.
  One preweighed *D. bellulus* was introduced to each test arena - after 24 and 48 h the test chambers were observed and the condition of the beetles was recorded. Beetles were deemed dead if there was no response to mechanical stimulation. If the beetle was a) unable to locomote in a normal manner b) positioned on it's dorsal side (i.e. on it's back with legs upwards) and was unable to right itself c) showed uncoordinated movement of appendages in response to stimulation, then it was said to be paralysed.
- After 48 h the pellet was removed, dried in an oven for 48 h at 45°C and the change in weight/weight of pellet consumed was calculated.
- Beetles were not re-used in subsequent experiments after exposure.

4. Results and Discussion

Raw data can be found in the Appendices.

The mean (± S.E.) fresh weight of beetles used in the tests was 6.436 ± 0.287 mg.

Figure 1 shows the response of the beetles to 24 and 48 h exposure to the two pellet treatments and a control. There was no mortality in the controls or the Multiguard® treatments after 48 h exposure. After 24 h 10 % of the beetles were paralysed in the Baysol® test - after a further 24 h there was 10 % mortality suggesting that there was no recovery from the paralysed state.

Figure 2 details the mean consumption of pellets during the 48 h exposure period, calculated from the difference in dry weight of the pellet before and after exposure. The estimated mean active ingredient (a.i.) intake was calculated from this as follows:

estimated active ingredient intake = change in dry weight of the pellet X the proportion of active ingredient in the pellet formulation

Multiguard® contains 90g of a.i. per kg of pellet
Baysol® contains 20g of a.i. per kg of pellet

In all cases there was a reduction in the mean dry weight of the pellets after 48 h exposure, therefore it can be assumed that *D. bellulus* was feeding on the pellets during the exposure. Beetles consumed more of the control pellets than the other formulations. There was only a small change in Baysol® pellet weights, however the mean intake of active ingredient (0.014 ± 0.005 mg) was clearly sufficient to effect mortality in 50 % of beetles after 48 h. There was higher mortality observed in Multiguard® than in the Baysol® treatments - this may be due to the relatively large calculated mean a.i. intake in the Multiguard® pellet test.

fig. 2. mean pellet consumption and estimated mean active ingredient (a.i.) ingested *D. bellulus* after 48 h exposure to 2 pellet formulations and a control.

treatment	mean change in pellet	estimated mean a.i.

	weight (mg) (± S.E)	consumption (mg) (± S.E)
control	8.837 ± 2.21	-
Multiguard®	14.221 ± 2.63	1.152 ± 024
Baysol®	7.314 ± 0.967	0.1463 ± 0.019

However, the data for the consumption of pellets should be used tentatively as an estimate of active ingredient actually consumed since in other tests performed (see other reports) it was concluded that using the change in dry weight as a measure of pellet consumption may not be an accurate method, especially for such small changes in weight. This would also lead to erroneous determination of the dose of active ingredient consumed, possibly explaining why there is no apparent trend between the dose of active ingredient consumed and the response observed (see Appendix 3 for raw data of a.i. intake and response).

5. Conclusions

D. bellulus displayed no adverse affects after 48 h exposure to blank control and Multiguard® pellets in laboratory trials. It can therefore be concluded that the Iron EDTA complex (the active ingredient against molluscs in Multiguard®) is not harmful to *this species* after 48 h in laboratory trials. A 10 % mortality was observed with Baysol® pellets after 48 h, therefore methiocarb (the active ingredient in Baysol®) is slightly toxic *D. bellulus*.

From the data obtained in this study it is concluded that of the 2 treatments tested, only Baysol® would cause *D. bellulus* mortalities in the field. Since there was only a low level of lethality (10 %) Baysol® applications would probably not seriously affect Melyrid populations although field experiments would need to be performed to confirm this. Results suggest Multiguard® should have no adverse effects on Melyrid beetles in areas where these pellets are broadcast.

It was not possible to determine the dose required to effect mortality since there was no apparent trend between active ingredient intake and response (see appendix 3). Using dry weight as an estimate of pellet consumption may not be accurate for such small changes in weight, as a result any estimate of active ingredient intake would also be inaccurate.

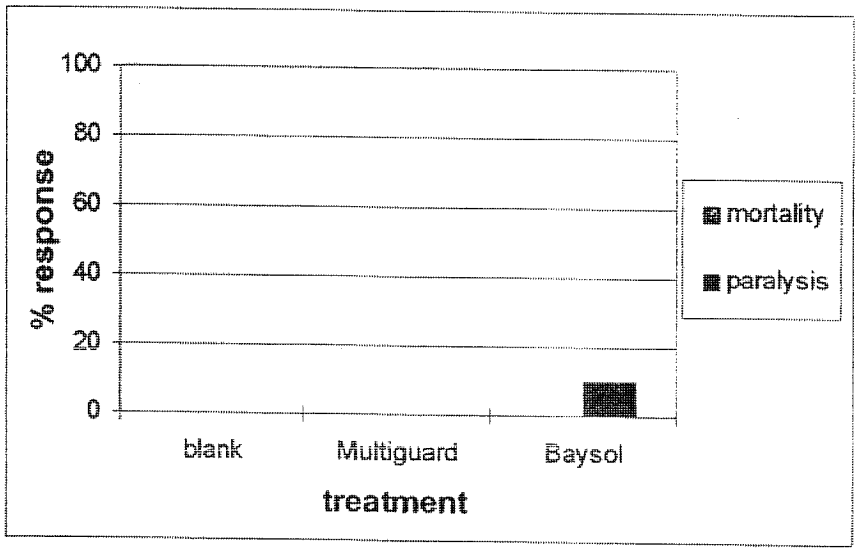
6. References

Pyke, B A and Brown, H (1996) The Cotton Pest and Beneficial Guide CRDC & CPTM 51pp.

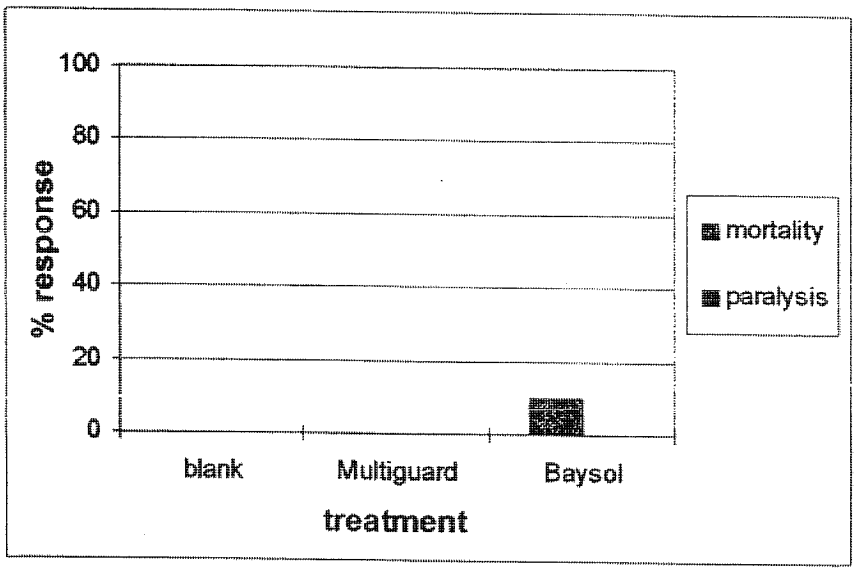
Lawrence, J F and Britton, E B (1991) Insects of Australia, 2nd Edition Vol II. CSIRO (Division of Entomology) Melbourne University Press pp 543-683.

fig. 1. *D. bellulus* response to 24 and 48 h exposure to the two pellet treatments and a control

24 h exposure



48 h exposure



APPENDIX 1

Raw Data for Melyrid beetle (*Dicranolauis bellulus*) tests

Individual beetle response after 24 and 48 h exposure to two pellet treatments and a control (O - beetle shows no adverse effects; X - beetle dead; P - beetle paralysed (see Protocol section for explanation of categorisation))

replicate	response					
	blank		Multiguard		Baysol	
	24 h	48 h	24 h	48 h	24 h	48 h
1	0	0	0	0	0	0
2	0	0	0	0	P	X
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0
% mortality	0	0	0	0	0	10
% paralysis	0	0	0	0	10	0

APPENDIX 2

Pellet consumption after 48 h exposure

treatment	weight before mg	weight after mg	weight change mg	% weight change
blank	86.031	60.200	25.831	30.025
	92.240	81.420	10.820	11.730
	83.740	70.040	13.700	16.360
	43.950	34.150	9.800	22.298
	71.670	65.600	6.070	8.469
	97.260	91.600	5.660	5.819
	61.220	58.650	2.570	4.198
	47.260	40.610	6.650	14.071
	74.980	69.550	5.430	7.242
	51.460	49.620	1.840	3.576
mean	**70.981**	**62.144**	**8.837**	**12.379**
S.E.	**6.07**	**5.57**	**2.21**	**2.7**

treatment	**weight before mg**	**weight after mg**	**weight change mg**	**% weight change**
Multiguard	205.380	196.000	9.380	4.567
	118.360	113.590	4.770	4.030
	124.420	111.920	12.500	10.047
	131.030	109.190	21.840	16.668
	109.480	101.630	7.850	7.170
	99.520	85.060	14.460	14.530
	n/a	n/a	n/a	n/a
	108.190	99.290	8.900	8.226
	118.320	94.720	23.600	19.946
	133.220	108.530	24.690	18.533
mean	**127.547**	**113.326**	**14.221**	**11.524**
S.E.	**10.4**	**10.8**	**2.47**	**2.01**

treatment	**weight before mg**	**weight after mg**	**weight change mg**	**% weight change**
Baysol	55.630	46.610	9.020	16.214
	48.510	42.560	5.950	12.266
	54.960	46.780	8.180	14.884
	61.400	51.720	9.680	15.765
	51.980	44.830	7.150	13.755
	49.310	43.390	5.920	12.006
	52.350	42.210	10.140	19.370
	48.370	48.360	0.010	0.021
	64.110	53.730	10.380	16.191
	41.950	35.240	6.710	15.995
mean	**52.857**	**45.543**	**7.314**	**13.647**
S.E.	**2.06**	**1.66**	**0.967**	**1.66**

APPENDIX 3

Estimated intake of active ingredient (a. i.) and corresponding response of each beetle after 24 and 48 h exposure to the test treatments.

The estimated intake of active ingredient is based on the change in dry weight of each pellet tested multiplied by the proportion of active ingredient in the pellet formulation (Multiguard® - 90g/kg; Baysol 20g/kg). The ratio of active ingredient to fresh body weight of each individual beetle is given as an indication of the dose of a. i. with body size. The beetle response to exposure is given after 24 and 48 h (O - beetle shows no adverse effects; X - beetle dead; P - beetle paralysed (see Protocol section for explanation of categorisation))

MULTIGUARD®

replicate	dry weight consumed (mg)	estimated a.i intake (mg)	body weight (mg)	ratio ai : body wt		response 24 h	response 48 h
1	9.380	0.84420	4.520	0.18677		0	0
2	4.770	0.42930	6.760	0.06351		0	0
3	12.500	1.12500	6.230	0.18058		0	0
4	21.840	1.96560	6.780	0.28991		0	0
5	7.850	0.70650	7.570	0.09333		0	0
6	14.460	1.30140	7.800	0.16685		0	0
7	0.000	0.00000	8.300	0.00000		0	0
8	8.900	0.80100	4.860	0.16481		0	0
9	23.600	2.12400	6.870	0.30917		0	0
10	24.690	2.22210	5.800	0.38312		0	0
mean	**12.799**	**1.1519**	**6.549**	**0.1838**	**% mortality**	0	0
s.e	**2.63**	**0.237**	**0.387**	**0.0371**	**% paralysis**	0	0

BAYSOL®

replicate	dry weight consumed (mg)	estimated a.i intake (mg)	body weight (mg)	ratio ai : body wt		response 24 h	response 48 h
1	9.020	0.18040	6.180	0.02919		0	0
2	5.950	0.11900	8.760	0.01358		P	X
3	8.180	0.16360	4.480	0.03652		0	0
4	9.680	0.19360	6.770	0.02860		0	0
5	7.150	0.14300	7.220	0.01981		0	0
6	5.920	0.11840	5.840	0.02027		0	0
7	10.140	0.20280	4.400	0.04609		0	0
8	0.010	0.00020	7.230	0.00003		0	0
9	10.380	0.20760	5.800	0.03579		0	0
10	6.710	0.13420	5.550	0.02418		0	0
mean	**7.314**	**0.1463**	**6.223**	**0.0254**	**% mortality**	**0**	**10**
s.e	**0.967**	**0.0193**	**0.42**	**0.0041**	**% paralysis**	**10**	**0**

APPENDIX A

Sublabel A:	**Agriculture**
Sublabel B:	**Turf & Ornamentals**
Sublabel C:	**Home & Garden**

SLUG & SNAIL KILLER

ACTIVE INGREDIENT:
Sodium Ferric Hydroxy EDTA.... 5.87%
OTHER INGREDIENTS:..... 94.13%
Total: 100.00%

EPA Reg. No. (Pending as 42697-AR)
EPA Est. No. #####-XX-###

Manufactured by:
Woodstream Corporation
69 North Locust Street
Lititz PA 17543

Sublabel A: Agriculture

[Alternate Brand Names: To be identified]

Notes to reviewer:
Designates optional wording- [text in brackets]
Designates comments to reviewer- *(italicized text)*

(FRONT PANEL)

SLUG & SNAIL KILLER

ACTIVE INGREDIENT:
Sodium Ferric Hydroxy EDTA.... 5.87%
OTHER INGREDIENTS:..... 94.13%
Total:..... 100.00%

KEEP OUT OF REACH OF CHILDREN

CAUTION

NET WEIGHT: XX LBS

EPA Reg. No. (Pending as 42697-AR) EPA Est. No. #####-XX-###

FIRST AID	
If In Eyes:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. Contact the nearest poison control center or 1-800-858-7378 for emergency medial treatment advice.	

PRECAUTIONARY STATEMENTS:
Hazards to Humans and Domestic Animals. **CAUTION:** Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling.

(BACK PANEL)

Personal Protective Equipment (PPE) Requirements: Applicators and other handlers must wear: long-sleeved shirt and long pants, waterproof gloves, and shoes plus socks. Follow manufacturer's instructions for cleaning/maintaining PPE. Keep and wash PPE separately from other laundry.

User Safety Recommendations: Users should wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

Environmental Hazards: For terrestrial uses: Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwaters or rinsate.

GENERAL INFORMATION: Slug & Snail Killer is attractive and deadly to slugs and snails. Slugs and snails quickly find and ingest the bait. This product has a non-toxic mode of action and can be used in areas where wildlife protection is a concern. The active ingredient in this product inhibits the transport of oxygen in slugs and snails. When snails & slugs ingest the bait, they stop feeding and crawl back to their shelter where they eventually die. Paralyzed or dead snails will rarely be seen in the open. This product will not degrade under damp & humid conditions when snails and slugs are most active. It remains effective under varying weather and environmental extremes.

DIRECTIONS FOR USE: It is a violation of Federal law to use this product in a manner inconsistent with its labeling. For any requirements specific to your State or Tribe, consult the State or Tribal agency responsible for pesticide regulation. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.

AGRICULTURAL USE REQUIREMENTS:
Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted entry intervals (REI). The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

There is a restricted entry interval of zero (0) hours for this product.

WHEN TO APPLY: Early morning or evening is the best time to apply this bait since slugs and snails travel and feed in the night or early morning. Reapply as the bait is consumed or at 14 day intervals. In areas of severe infestation, apply bait more heavily.

WHERE TO APPLY: All likely areas of infestation should be treated, especially around the perimeter of fields because slugs and snails travel into plant areas from daytime refuge. Apply bait around the base of plantings that show visible damage. Slugs and snails favor damp places around and within vegetable plots like beans, tomatoes, lettuce, cabbage, celery and squash, and in weeds or ditches around field margins.

HOW TO APPLY:
Slug & Snail Killer should be applied evenly to the soil around or near plants and areas to be protected. Do not place bait in heaps. Apply while the ground is moist, in early morning, late evening, or after rainfall. The soil should be moist, but with little or no standing water. Apply using conventional ground spreader application equipment, or using a handheld spreader or rotary type spreader, or by dispensing directly from container to smaller areas as spot treatment. Carefully read and follow the instructions for the type of spreader you use.

APPLICATION RATES

Crop/Use	Rate	Directions
Vegetables including (but not limited to): asparagus, artichokes, beans, beets, blackeyed peas, broccoli, Brussels sprouts, cabbage, cantaloupe, carrots, cauliflower, corn, cucumbers, eggplants, garlic, lettuce, onions, peas, peppers, potatoes, radishes, rutabagas, spinach, squash, Swiss chard, tomatoes, turnips.	20-40 lbs per acre	Scatter the bait around the perimeter of vegetables and between rows. Apply at lower rate if infestation is low and pellets can be applied near localized infestation. For heavier infestations, apply at higher rate. Reapply every 14 days.
Orchard Fruits including (but not limited to): apples, avocados, apricots, cherries, grapes, melons, peaches, plums, nectarines, citrus, and pears	20-40 lbs per acre	For mature trees in orchards, scatter bait evenly around the base of the trees. Use higher rates for heavy infestations. For new seedlings, spread bait evenly around the base of the stem using a Teaspoon.
Berries including (but not limited to): strawberries, blackberries, blueberries, boysenberries, loganberries, and raspberries.	20-40 lbs per acre	Scatter by hand or with granular spreaders around the perimeter of plot and between rows. Do not broadcast entire area, but treat selectively. For small plots, treat around the base of the plants to be protected. Use higher rate for heavy infestations.
Field Crops including (but not limited to): beans, field corn, sweet corn, soybeans, sugarbeets, sugar	20-40 lbs per acre	At all stages of growth, apply the bait between the rows and around the field perimeter. Use higher rate for heavy infestations.

cane, asparagus, artichokes, beets, broccoli, Brussels sprouts, cabbage, carrots, cauliflower, cucumbers, lettuce, onions, peas, peppers, potatoes, radishes, strawberries, tomatoes, and turnips.		
Vineyards	20-40 lbs per acre	Scatter bait evenly at the base of vines and between rows. Use higher rates for heavy infestations.
Greenhouse grown vegetables	½ TSP per 9 inch pot	Scatter bait evenly on the soil surface of potted plants being damaged by slugs. Bait can also be scattered around pots on greenhouse benches.
Grass Grown for Seed Production, Wheat	20-40 lbs per acre	Scatter bait with a granular spreader in areas where slugs or snails are observed.

STORAGE AND DISPOSAL:
Do not contaminate water, food or feed by storage and disposal.
Pesticide Storage: Store in original, closed container, in a cool dry place. Do not store in direct sunlight.
Pesticide Disposal: Wastes resulting from use of this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal: Completely empty package into application equipment. Then dispose of empty package in trash, in a sanitary landfill or by incineration, or, if allowed by State and local authorities, by burning. If burned, stay out of smoke.

Manufactured by:
Woodstream Corporation
69 North Locust Street
Lititz PA 17543
800-800-1819 For Information

(OPTIONAL MARKETING CLAIMS)

- For control of snails and slugs.
- Protects plants from snails & slugs.
- Patent No. PCT/AU97/00033
- Patented technology.
- Patented snail & slug killer.
- 1 lb treats up to 2000 square feet.
- Remains effective after rain or watering
- Effective under wet & dry conditions
- Convenient. Easy-to-Use. Requires no mixing, spraying or special applicators.
- For use around vegetables, fruit trees, citrus, berries, ornamentals, lawns, and in greenhouses.
- Highly attractive
- Killer [Kills]
- Kills Snails & Slugs
- Stops snails & slugs from feeding
- Lures slugs & snails from plants and hiding places
- Protects plants from snail & slug damage
- Does not contain metaldehyde which is toxic to dogs and wild animals
- Metaldehyde-free

Sublabel B: Turf & Ornamentals

[Alternate Brand Names: To be identified]

Notes to reviewer:
Designates optional wording- [text in brackets]
Designates comments to reviewer- *(italicized text)*

(FRONT PANEL)

SLUG & SNAIL KILLER

ACTIVE INGREDIENT:
Sodium Ferric Hydroxy EDTA.... 5.87%
OTHER INGREDIENTS:..... 94.13%
Total:..... 100.00%

KEEP OUT OF REACH OF CHILDREN

CAUTION

NET WEIGHT: XX LBS

EPA Reg. No. (Pending as 42697-AR) EPA Est. No. #####-XX-###

FIRST AID	
If In Eyes:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. Contact the nearest poison control center or 1-800-858-7378 for emergency medial treatment advice.	

PRECAUTIONARY STATEMENTS:
Hazards to Humans and Domestic Animals. **CAUTION:** Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling.

(BACK PANEL)

Personal Protective Equipment (PPE) Requirements: Applicators and other handlers must wear: long-sleeved shirt and long pants, waterproof gloves, and shoes plus socks. Follow manufacturer's instructions for cleaning/maintaining PPE. Keep and wash PPE separately from other laundry.

User Safety Recommendations: Users should wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

Environmental Hazards: For terrestrial uses: Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwaters or rinsate.

GENERAL INFORMATION: Slug & Snail Killer is attractive and deadly to slugs and snails. Slugs and snails quickly find and ingest the bait. This product has a non-toxic mode of action and can be used in areas where wildlife protection is a concern. The active ingredient in this product inhibits the transport of oxygen in slugs and snails. When snails & slugs ingest the bait, they stop feeding and crawl back to their shelter where they eventually die. Paralyzed or dead snails will rarely be seen in the open. This product will not degrade under damp & humid conditions when snails and slugs are most active. It remains effective under varying weather and environmental extremes.

DIRECTIONS FOR USE: It is a violation of Federal law to use this product in a manner inconsistent with its labeling. For any requirements specific to your State or Tribe, consult the State or Tribal agency responsible for pesticide regulation. Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.

AGRICULTURAL USE REQUIREMENTS:
Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted entry intervals (REI). The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

There is a restricted entry interval of zero (0) hours for this product.

NON-AGRICULTURAL USE REQUIREMENTS
The requirements in this box apply to uses of this product that are **not** within the scope of the Worker Protection Standard for agricultural pesticides (40 CFR Part 170). The WPS applies when this product is used to produce agricultural plants on farms, forests, nurseries or

greenhouses.

Keep unprotected persons out of treated areas until dusts have settled.

WHEN TO APPLY: Early morning or evening is the best time to apply this bait since slugs and snails travel and feed in the night or early morning. Reapply as the bait is consumed or at 14 day intervals. In areas of severe infestation, apply bait more heavily.

WHERE TO APPLY: All likely areas of infestation should be treated, especially around the perimeter of lawns, landscaped areas and nursery fields because slugs and snails travel into plant areas from daytime refuge. Apply bait around the base of plantings that show visible damage. Slugs and snails favor damp places around and within plants, and in weeds or ditches around field margins.

AMOUNT TO APPLY: (TSP=Teaspoon)
Spot Treatment- 1 TSP per square yard.
Broadcast Treatment- 2 to 3 cups per 1000 square feet. 1 lb. per 2000 square feet.

HOW TO APPLY: Slug & Snail Killer should be applied evenly to the soil around or near plants and areas to be protected. Do not place bait in heaps. Apply while the ground is moist, in early morning, late evening, or after rainfall. The soil should be moist, but with little or no standing water. Apply using conventional ground spreader application equipment, or using a handheld spreader or rotary type spreader, or by dispensing directly from container to smaller areas as spot treatment. Carefully read and follow the instructions for the type of spreader you use.

APPLICATION RATES

Use	Rate	Directions
Outdoor Ornamentals	1 lb. per 2000 square feet	Use a teaspoon to evenly scatter bait in a 6-inch wide circular band around the base of the plants to be protected. Do not allow bait to form heaps.
Indoor Container- Greenhouses	½ TSP per 9 inch pot	Scatter bait evenly on the soil surface of potted plants being damaged by slugs. Bait can also be scattered around pots on greenhouse benches.
Outdoor Container-Grown Nursery Plants	1 TSP per container	Scatter bait evenly on the soil surface of containers or scatter evenly around the soil near containers.
Turf, golf courses, sod farms	1 lb. per 2000 square feet	Scatter bait with a granular spreader in areas where slugs or snails are observed.

Manufactured by:
Woodstream Corporation
69 North Locust Street
Lititz PA 17543 800-800-1819 For Information

(OPTIONAL MARKETING CLAIMS)

- For control of snails and slugs.
- Protects plants from snails & slugs.
- Patent No. PCT/AU97/00033
- Patented technology.
- Patented snail & slug killer.
- 1 lb treats up to 2000 square feet.
- Remains effective after rain or watering
- Effective under wet & dry conditions
- Convenient. Easy-to-Use. Requires no mixing, spraying or special applicators.
- For use around ornamentals, lawns, and landscaped areas.
- Highly attractive
- Killer [Kills]
- Kills Snails & Slugs
- Stops snails & slugs from feeding
- Lures slugs & snails from plants and hiding places
- Protects plants from snail & slug damage
- Does not contain metaldehyde which is toxic to dogs and wild animals
- Metaldehyde-free

Sublabel C: Home & Garden

[Alternate Brand Names: To be identified]

Notes to reviewer:
Designates optional wording- [text in brackets]
Designates comments to reviewer- *(italicized text)*

(FRONT PANEL)

SLUG & SNAIL KILLER

ACTIVE INGREDIENT:
Sodium Ferric Hydroxy EDTA.... 5.87%
OTHER INGREDIENTS:..... 94.13%
Total:..... 100.00%

KEEP OUT OF REACH OF CHILDREN
CAUTION (See back [side] [other] panel for additional precautions & first aid.)

EPA Reg. No. (Pending as 42697-AR)
EPA Est. No. #####-XX-###
NET CONTENTS: XX LBS
(Package filled by weight and not to capacity)

(BACK PANEL)

PRECAUTIONARY STATEMENTS:
Hazards to Humans and Domestic Animals. **CAUTION:** Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco.

FIRST AID	
If In Eyes:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. Contact the nearest poison control center or 1-800-858-7378 for emergency medial treatment advice.	

Environmental Hazards: Do not contaminate water when disposing of equipment washwaters or rinsate.

Carefully read and understand this pesticide label before use!

GENERAL INFORMATION: Slug & Snail Killer is attractive and deadly to slugs and snails. Slugs and snails quickly find and ingest the bait. This product has a non-toxic mode of action and can be used in areas where pet and wildlife protection is a concern. The active ingredient in this product inhibits the transport of oxygen in slugs and snails. When snails & slugs ingest the bait, they stop feeding and crawl back to their shelter where they eventually die. Paralyzed or dead snails will rarely be seen in the open. This product will not degrade under damp & humid conditions when snails and slugs are most active. It remains effective under varying weather and environmental extremes. Slug & Snail Killer can be used effectively around seedlings, potted plants, vegetables, perennials and annuals. 1 lb will treat approximately 2000 square feet.

DIRECTIONS FOR USE: It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

WHEN TO APPLY: Evening is the best time to apply this bait since slugs and snails travel and feed in the night or early morning. Reapply as the bait is consumed or at 14 day intervals. In areas of severe infestation, apply bait more heavily.

WHERE TO APPLY: On Lawns, Perimeter of Home, Around Ornamental Plants (flowers, shrubs & trees) and in Home Gardens. All likely areas of infestation should be treated, especially around the perimeter of garden plots because slugs and snails travel into plant areas from daytime hiding places. Apply bait around the base of plantings that show visible damage. Do not place bait in heaps. Immediately clean up any spilled bait. Slugs and snails favor damp places around vegetable plants such as beans, tomatoes, lettuce, cabbage, celery and squash. Slugs and snails often seek shelter inside large leafy plantings like hostas & dahlias. Other favorite areas are flower gardens,

rock gardens, hedges, dichondra lawns, citrus groves, ivy patches, and other ground cover where they obtain shelter by day.

AMOUNT TO APPLY: (TSP=Teaspoon)
Spot Treatment- 1 TSP per square yard.
Broadcast Treatment- 2 to 3 cups per 1000 square feet. 1 lb. per 2000 square feet.

HOW TO APPLY:
Do not use kitchen utensils for measuring!
Keep measuring utensils with pest control products out of reach of children. Slug & Snail Killer should be applied evenly to the soil around or near plants and areas to be protected. Apply in early morning or late evening when soil is moist. If the ground is dry, wet it before applying the bait. The soil should be moist, but with little or no standing water. Avoid creating piles of the bait. For broadcast application, standard broadcast spreaders may be used. For row application, standard granular spreaders may be used. Product may be dispensed directly from container to smaller areas as spot treatment or can be applied using a handheld spreader. Carefully read and follow the instructions for the type of spreader you use.

STORAGE AND DISPOSAL:
Storage: Store in a cool dry place, out of reach of children and domestic animals. Store in original container only.
If Empty: Do not reuse this container, place in trash or offer for recycling if available.
If Partly Filled: Call your local solid waste agency or 1-800-CLEANUP for disposal instructions. Never place unused product down any indoor or outdoor drain.

Manufactured by:
Woodstream Corporation
69 North Locust Street
Lititz PA 17543
800-800-1819 For Information

Slug & Snail Killer – MASTER LABEL
EPA Reg. No. (Pending as 42697-AR)
Label version (3) dated March 13, 2006
Page 14 of 14

(OPTIONAL MARKETING CLAIMS)

- Protects plants from snails & slugs.
- May be used in areas where there are dogs, cats, & birds.
- Garden Friendly- Breaks down into nutrients for plants and soils.
- For use by people who care about their pets, native birds, fauna & earthworms.
- Patent No. PCT/AU97/00033
- Patented technology.
- Patented snail & slug killer.
- 1 lb treats up to 2000 square feet.
- Remains effective after rain or watering
- Effective under wet & dry conditions
- Convenient. Easy-to-Use. Requires no mixing, spraying or special applicators.
- Can be used in vegetable gardens.
- For use around gardens, flowers, shrubs, trees, and lawns.
- Can be used around domestic animals [pets] and wildlife
- Highly attractive
- Killer [Kills]
- Kills Snails & Slugs
- For home and garden use
- Stops snails & slugs from feeding
- Lures slugs & snails from plants and hiding places
- Protects plants from snail & slug damage
- Does not contain metaldehyde which is toxic to dogs and wild animals
- Metaldehyde-free

CBI-deleted

Appendix B

ALL PAGES OF APPENDIX B (PAGES 1 THROUGH 10) HAVE BEEN CBI-DELETED

Appendix C

ALL PAGES OF APPENDIX C (PAGES 1 THROUGH 123) HAVE BEEN CBI-DELETED

ALL PAGES OF APPENDIX D (PAGES 1 THROUGH 71) HAVE BEEN CBI-DELETED

From: Origin ID: (717)626-2125
David Anderson
Woodstream Corporation
69 North Locust Street

Lititz, PA 17543

FedEx Express

Ship Date: 12APR06
ActWgt: 8 LB
System#: 4254061/INET2400
Account#: S *********

REF:

Delivery Address Bar Code

SHIP TO: (202)720-3252 BILL SENDER

Robert Pooler
USDA/AMS/TM/NOP
1400 Independence Ave. S.W.

Washington, DC 20250

STANDARD OVERNIGHT

THU
Deliver By:
13APR06

TRK# 7903 9166 3870 FORM 0201

IAD A2

20250 -DC-US

19 WASA

Shipping Label: Your shipment is complete

1. Use the 'Print' feature from your browser to send this page to your laser or inkjet printer.
2. Fold the printed page along the horizontal line.
3. Place label in shipping pouch and affix it to your shipment so that the barcode portion of the label can be read and scanned.

Warning: Use only the printed original label for shipping. Using a photocopy of this label for shipping purposes is fraudulent and could result in additional billing charges, along with the cancellation of your FedEx account number.

Use of this system constitutes your agreement to the service conditions in the current FedEx Service Guide, available on fedex.com. FedEx will not be responsible for any claim in excess of $100 per package, whether the result of loss, damage, delay, non-delivery, misdelivery, or misinformation, unless you declare a higher value, pay an additional charge, document your actual loss and file a timely claim. Limitations found in the current FedEx Service Guide apply. Your right to recover from FedEx for any loss, including intrinsic value of the package, loss of sales, income interest, profit, attorney's fees, costs, and other forms of damage whether direct, incidental, consequential, or special is limited to the greater of $100 or the authorized declared value. Recovery cannot exceed actual documented loss. Maximum for items of extraordinary value is $500, e.g. jewelry, precious metals, negotiable instruments and other items listed in our Service Guide. Written claims must be filed within strict time limits, see current FedEx Service Guide.