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Iron EDTA for Food Fortification

A REPORT OF THE
INTERNATIONAL NUTRITIONAL ANEMIA
CONSULTATIVE GROUP

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About INACG

The purpose of the International Nutritional Anemia Consultative Group (INACG) is to guide international activities aimed at reducing nutritional anemia in the world.

INACG works through a steering committee chaired by Dr. Richard C. Theuer (Beech-Nut Nutrition Corporation). Other members are Dr. Rodolfo Florentino (Food and Nutrition Research Institute, Philippines), Dr. Samuel G. Kahn (AID), Dr. Louis W. Sullivan (Morehouse Medical School), Dr. Fernando Viteri (University of California), and Dr. Tomas Walter (University of Chile). INACG offers consultation and guidance to various operating and donor agencies who are seeking to reduce iron deficiency and other nutritionally preventable anemias. As part of this service, INACG has prepared guidelines and recommendations for:

- Assessing the regional distribution and magnitude of nutritional anemia;
- Developing intervention strategies and methodologies to combat iron deficiency anemia;
- Evaluating the effectiveness of implemented programs on a continuing basis so that evaluation of the effectiveness of intervention techniques is a continuing and dynamic process; and
- Research needed to support the assessment, intervention, and evaluation of programs.

INACG is dedicated to reducing the prevalence of iron deficiency and other nutritionally preventable anemias worldwide. In fulfilling this mandate, INACG sponsors scientific reviews and convenes task force groups to analyze issues related to etiology, treatment, and prevention of nutritional anemias. The need to examine these issues is acknowledged as being important to the establishment of public policy and action programs. A series of monographs and reports are the result of these task force groups' efforts:

- *Guidelines for the Eradication of Iron Deficiency Anemia* (1977)
- *Iron Deficiency in Infancy and Childhood* (1979) (available in English, French, and Spanish)
- *Iron Deficiency in Women* (1981) (available in English, French, and Spanish)
- *Iron Deficiency and Work Performance* (1983)
- *Design and Analysis of Iron Supplementation Trials* (1984)
- *Measurements of Iron Status* (1985)
- *Combating Iron Deficiency in Chile: A Case Study* (1986) (available in English, French, and Spanish)
- *Guidelines for the Control of Maternal Nutritional Anemia* (1989) (available in English, French, and Spanish)
- *Combating Iron Deficiency Anemia Through Food Fortification Technology: An Action Plan* (1992) (available in English, French, and Spanish)

Single copies of these reports are available free of charge to developing countries and for $3.50 (U.S.) to developed countries. Copies are available from the INACG Secretariat:

INACG Secretariat
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Preface

Iron deficiency anemia (IDA) is the most prevalent nutritional problem in the world today. At greatest risk are young children and women of reproductive age, especially pregnant and lactating women. Though prevalent in all countries, IDA is most widespread and severe in developing countries. The cause is due in large part to the types of foods consumed, diets principally of high cereals, legumes and vegetables. Often these foods contain substances, such as phytates, which inhibit the absorption and biological utilization of iron.

Significant progress has been made in understanding the wide variations in iron bioavailability as affected by the form of chemical iron, the food and diet consumed, and the fortification technology for a variety of foods, with different forms of iron. Major studies have been conducted with sodium iron ethylenediaminetetraacetic acid (NaFeEDTA). NaFeEDTA is a unique compound that both makes iron available for absorption in the presence of several food inhibitory factors, and can be incorporated into certain foods without adverse organoleptic effects, whether taste, smell or color. It represents a major addition to the iron arsenal because this unique iron compound can be used in diets where conventional food iron compounds cannot—high cereal or legume diets.

This monograph presents the scientific and technical information that paved the way for the Joint (FAO/WHO) Expert Committee on Food Additives (JECFA) to recommend that NaFeEDTA be included as an iron food additive by the Codex Alimentarius Commission of the World Health Organization (WHO) and Food and Agriculture Organization (FAO). The monograph is intended for use by industry, donor agencies, governments, nongovernment organizations, and research institutions.

The U.S. Agency for International Development, long a supporter of the International Nutritional Anemia Consultative Group (INACG), believes this monograph will significantly advance prospects for appropriate and effective iron fortification programs. It is an example of the Agency's effort to alleviate iron deficiency anemia worldwide.

Richard M. Seifman
Director
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Bureau for Research and Development
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Acknowledgments

The International Nutritional Anemia Consultative Group (INACG) wishes to thank the authors of this monograph for their tireless efforts to ensure the completion of their task. They gave their time both at home and in meetings far from their homes. The publication of this monograph will, we hope, reward their efforts by encouraging the use of iron EDTA as an effective and safe means of improving iron status in populations around the world.

INACG also wishes to thank the members of the Editorial Review Board for their active participation in reviewing the monograph and for their constructive suggestions for improving the document. The INACG Secretariat is to be commended for their efforts in keeping the publication schedule on track and for their efforts in expediting the submission of the necessary information to the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In particular, INACG wishes to thank Ms. Carolyn Darrehmane and Ms. Molly Hovermale for their efforts in keeping up with all of the correspondence and details that arise in a project such as this one.

We are indebted to Ms. Judith Dickson for her expertise in copy editing the manuscript and to Mr. Walter Ludwig for design ideas. We believe their attention to detail has produced a well-organized and visually pleasing document.

Finally and perhaps most importantly, INACG wishes to acknowledge the financial support given to this project by the U.S. Agency for International Development. Without this support and the constant encouragement of Dr. Samuel Kahn this monograph would not have been published.

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Executive Summary

Summary Statement: *Sufficient information is available to recommend the use of sodium iron ethylenediaminetetraacetic acid (NaFeEDTA), also called iron EDTA, for food fortification in programs to improve iron status. This recommendation is supported by the recent provisional approval by the Joint FAO/WHO Expert Committee on Food Additives of NaFeEDTA as safe in food fortification programs for populations in which iron deficiency is endemic.*

Fortification with NaFeEDTA is expected to be most efficacious in iron-deficient populations subsisting on cereal- and legume-based diets, such as whole-wheat flour, corn meal, soybean, and other legume products. Condiments such as sugar, salt, fish sauce, soy sauce, and certain spices could also serve as vehicles for delivering NaFeEDTA.

It is also possible to use, when appropriate, other soluble iron salts in conjunction with sodium EDTA or sodium calcium EDTA as alternatives to NaFeEDTA. This less expensive technology, however, is useful only for foods stored for short periods. These compounds disintegrate relatively rapidly, and as they do, they render foods organoleptically unsuitable.

Thus, NaFeEDTA is particularly attractive for food fortification because of its chemical stability and suitability in foods that require prolonged storage or that are prepared at high temperatures. This is in addition to the unique iron bioavailability of the compound, its property of not being incapacitated by many food iron inhibitors, and its safe nature as indicated from toxicological studies, human absorption tests, and field fortification trials.

About 1 billion of the world's 6 billion inhabitants suffer from nutritional iron deficiency. It is the most common cause of anemia. Young children and women of childbearing age are affected most. The consequences vary depending on the setting in which it occurs. Anemia during pregnancy leads to an increased risk of premature labor and a higher perinatal morbidity and mortality. The mental development of young children may be impaired and later affect their performance at school. Iron-deficient adults cannot carry out physical endurance tasks as well as their iron-replete peers, limiting their ability to generate income in many developing countries.

Nutritional iron deficiency results from the failure of iron absorbed from the diet to meet body requirements. It occurs most commonly when needs are increased by rapid growth, menstruation, pregnancy, or pathological blood loss. Dietary iron intake always exceeds requirements by a sizable margin. Iron deficiency is, therefore, the result of inadequate absorption owing to the body's inability to extract all the iron needed from many foods. While meals with a large meat component are an excellent source of iron, many cereal and vegetable foods contain large quantities of phytate, polyphenols, and other constituents that bind iron, rendering it unavailable for absorption.

Many attempts have been made to reduce the prevalence of iron deficiency by fortifying foods with iron. Unfortunately, bioavailable iron salts added to foods are affected by the same inhibitory factors as the iron already present in the food. In the few trials that have proven to be effective, iron has been added in combination with an absorption enhancer. The use of vitamin C and iron in milk was effective in Chilean children. Satisfactory results were reported for the use of ferrous sulfate stabilized with orthophosphoric acid and sodium acid sulfate and added to table salt. From the practical point of view, both strategies have drawbacks. Vitamin C is expensive and relatively unstable. Prolonged storage or cooking frequently renders it inactive. The use of iron-fortified salt is limited by the lack of central distribution points in many counties and the susceptibility of ferrous sulfate to inhibitory food factors.

A third strategy, the use of sodium iron ethylenediaminetetraacetic acid (NaFeEDTA), is the subject of this monograph. NaFeEDTA is particularly attractive because of its chemical stability. It is suitable as a fortificant for foods that require prolonged storage or high temperatures during preparation.

The physical properties and physiological effects of ethylenediaminetetraacetic acid (EDTA) are discussed in chapter II. EDTA is a multidentate (literally "multitoothed") chelating agent capable of forming complexes with metal ions such as calcium, iron, copper, manganese, lead, etc. The nature of the solution in which EDTA is present with respect to factors such as acidity and the concentrations of the various metal ions determines which metal complex is formed; e.g., in the acidic environment present in the human stomach, binding of iron to EDTA is favored. However, when the intestinal contents become more alkaline in the duodenum because of the inflow of bile and pancreatic juices, the iron is exchanged in part for calcium, copper, and zinc. In human beings, most iron is absorbed from the duodenum. Therefore, EDTA promotes iron absorption by acting as a "shuttle," freeing iron in the stomach from factors such as phytate that inhibit its absorption and then releasing the iron in the proximal end of the duodenum, where it is absorbed.

The amount of iron taken up by the body varies depending on the body's requirements. Only a small fraction of the EDTA itself (5% or less) is absorbed, most of it in a form in which it is bound to metal ions other than iron. Absorbed EDTA is rapidly and completely excreted in the urine and does not accumulate in the body. In the dose range recommended for fortification, it is nontoxic. In fact similar quantities of EDTA have been present in the diets of individuals in many countries for many years as a result of its use as a sequestering agent in foods to prevent changes in color, flavor, and texture. The "shuttle" action described above for EDTA occurs regardless of whether EDTA is introduced as NaFeEDTA or another EDTA salt such as Na_2EDTA. However, its effectiveness is critically dependent on the ratio of the quantity of iron to the quantity of EDTA present in the food. Iron is best absorbed when the iron:EDTA ratio (expressed as a molar ratio) is between 4:1 and 1:1. Further increases in the proportion of EDTA reduce iron absorption.

It is clear from the preceding discussion that iron and EDTA are absorbed by separate and independent mechanisms when NaFeEDTA is added to a meal. Less than 1% of the NaFeEDTA complex is absorbed intact. This small amount is completely

excreted in the urine. The iron is not utilized by the body.

Studies of the effects of NaFeEDTA and Na_2EDTA on human iron absorption are described in chapter III. Iron added as NaFeEDTA is absorbed two to three times better than ferrous sulfate from vegetable meals. The difference in absorption is greatest in meals with poor ferrous sulfate bioavailability. From the practical point of view, it is important to note that at least 10% of the fortification of iron is absorbed from all but the most inhibitory meals. The use of the EDTA chelator has an additional benefit that is a predictable result of its action as an iron "shuttle" between iron binders in the food and the absorption process in the duodenum. It improves the absorption of the intrinsic food iron as well. As a consequence, at least 10% of all the iron (both food iron and fortification iron) is absorbed from the meal. Furthermore, fortification with iron and EDTA is equally effective when presynthesized NaFeEDTA is added to a food, as when a soluble iron salt such as ferrous sulfate and Na_2EDTA is added separately to the same meal provided that adequate mixing occurs.

The absorption of iron from meals fortified with NaFeEDTA is controlled by the same physiological factors that determine food iron absorption. There is a close inverse relationship between the percentage of iron absorbed and the size of body iron stores, providing further support for the contention that NaFeEDTA iron is available to physiological pathways for absorption.

NaFeEDTA is not an appropriate fortificant for meals in which iron bioavailability is intrinsically satisfactory or has been improved by the addition of another absorption promoter such as ascorbic acid. Bioavailability is not improved by the EDTA. In fact, the promoting effect of ascorbic acid may be diminished and the percentage of iron absorption reduced by EDTA.

The final chapter (chapter IV) describes the results of three field trials employing NaFeEDTA. The vehicles used were sugar in one and condiments in the other two. Wheat flour and other cereals are equally suitable potential vehicles for NaFeEDTA. Although NaFeEDTA is not free of organoleptic problems, it performs better than many other compounds. It did not affect the consumer acceptability of the products to which it was added in the three trials. An improvement in iron status among the groups receiving the fortified food was noted in each of the trials. In the more recent trial, the observed prevalence of iron deficiency among women dropped from 22 to 4.9% at the end of the 2-year fortification. Those with anemia accumulated about half a gram of body iron. In contrast, iron-replete males experienced only a small rise in iron stores. No untoward effects were observed, and no change in zinc status was noted in the two trials in which plasma and urinary zinc levels were measured. In this context, it is important to note that despite earlier concerns about the effect of EDTA on zinc, recent human studies suggest that the EDTA level proposed for iron fortification would either have no effect on zinc balance or improve zinc balance from diets with a very low-zinc content.

Based on the information presented in this monograph, it is evident that EDTA is a safe food additive. Na_2EDTA and $CaNa_2EDTA$ have been used by the food industry for a considerable period of time. NaFeEDTA would be suitable for fortifying wheat flour, other cereal and legume products, and many other foods. Its use should be

targeted at adults and children over the age of 2 years. Provisional approval for its use in monitored national fortification programs has been obtained from the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This committee serves in an advisory capacity to the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Codex Alimentarius Commission.

I. Introduction

Prevalence of Iron Deficiency Anemia

It has been estimated that 1 billion of the world's 5 billion inhabitants suffer from nutritional iron deficiency anemia (MacPhail and Bothwell, 1992). Most of these individuals live in developing countries where the prevalence of iron deficiency anemia may be as high as 60-70% in preschool children and 60-80% in pregnant women (Bothwell et al., 1979; Simmons and Gurney, 1980). Some survey data are at least 20 years old, but current estimates confirm that 60-70% of the most vulnerable individuals in some countries are still affected despite 40 years of research (INACG, 1990; MacPhail and Bothwell, 1992).

Consequences of Iron Deficiency

The physiologic and economic consequences of iron deficiency are well established. They occur at all ages although the most significant effects on the individual may vary depending on developmental stage. Iron deficiency is the most important cause of anemia during pregnancy, and even a modest decrease in hematocrit may double the risk of premature labor (Lieberman et al., 1988). Perinatal morbidity and mortality are increased (Bothwell et al., 1979). In growing children iron deficiency may significantly limit intellectual potential (Lozoff et al., 1991); and psychomotor development can be affected permanently. Iron deficiency in childhood may therefore have significant long-term consequences for the lifestyles and achievement potentials of those affected. One study recorded an improved rate of physical growth and diminished severity of acute and chronic infectious diseases in iron-deficient anemic children given an oral iron supplement (Chwang et al., 1988).

From the socioeconomic point of view, the limitation that iron deficiency places on an adult's ability to perform tasks requiring physical endurance may be equally important. Studies involving laborers in different countries have demonstrated impaired productivity as a result of iron deficiency (Edgerton et al., 1979; Basta et al., 1979). Affected individuals are usually the family breadwinners. Consequently the economic impact of iron deficiency on the family and the society may be far greater than is immediately apparent (Levin, 1986).

In summary, from the public health point of view, iron deficiency places a severe handicap on the productivity of society because of the long-term consequences of premature birth, impaired cognitive achievement, and reduced ability to perform physically demanding tasks.

Causes of Nutritional Iron Deficiency

Nutritional iron deficiency results from the failure of iron absorbed from the diet to meet body requirements. Therefore, it is most often associated with periods of increased physiological need. The highest prevalence rates occur in infants, children, and adolescents, reflecting the demands of growth and the expanding red cell mass. Rates are also high in premenopausal women because of menstrual blood losses and the increased demands of pregnancy (Bothwell et al., 1979). Nutritional iron deficiency is aggravated when there are pathological losses, such as with hookworm infestation.

On the basis of measurements taken in Western countries, dietary iron intake is closely related to energy intake (approximately 6 mg Fe/1000 kcal; Bothwell et al., 1979). Relative differences between iron and caloric requirements in men, women, and children are reflected by significant variation in the percentage of dietary iron that must be absorbed to meet physiological needs (Table 1). Adult men, who have the highest caloric intakes and lowest iron requirements, are least likely to suffer from iron deficiency, because absorption of as little as 5% of the iron ingested may satisfy their needs. Infants in the postweaning period require fewer calories but almost as much iron. Women with high physiological losses require more iron but fewer calories. The latter two groups must extract approximately three times as much of the iron they consume (13, 16% respectively) and therefore are at much greater risk when the bioavailability of dietary iron is low. Pathological blood loss would further compromise iron balance.

It is also evident from Table 1 that daily iron intake always exceeds the body's requirement by a sizeable margin. If all the ingested iron were available for absorption, iron deficiency would not occur. However, even when conditions are most favorable

Table 1 Relationship Between Iron Requirements and Dietary Iron Supply

	Young children	Adults	
	6–18 Months	Male	Female
Mean weight (kg)	10	70.0	60.0 (60.0)
Food intake (kcal)	1120	3000	2300
Iron intake (mg)	6.7	19.2	13.8 (13.8)
Iron requirement (mg)	0.9	1.0	1.4 (2.2)
Absorption requirement (%)	13	5.0	10.0 (16.0)

Values in parentheses are for women with monthly menstrual blood loss exceeding 80 mL (10% of women).
Source: *Encyclopaedia of Food Science*, Food Technology & Nutrition

for iron absorption, as when an iron-deficient individual eats a meal providing highly bioavailable iron, only about 30% of the iron is absorbed (Cook, 1990); more than two thirds is wasted. This is a consequence of the body's inability to extract needed iron from food and not of physiological control mechanisms. Absorption of the most available form of iron (ferrous sulfate together with ascorbic acid given in the fasting state to an iron-deficient individual) may exceed 90% (Bezwoda et al., 1979). Observations like these have led to the realization that the bioavailability of dietary iron is as important or even more important in the maintenance of iron balance than is the total amount ingested.

Iron Absorption

Several excellent reviews describing the factors governing the bioavailability of food iron as well as the adaptations that occur in iron deficiency are available (Hallberg, 1981; Charlton and Bothwell, 1983; Monsen, 1988; Cook, 1990). Only a brief summary will be given here. Iron enters the body through two separate pathways: heme and nonheme.

Heme iron. Heme iron derived primarily from hemoglobin and myoglobin in meat is transferred to the intestinal cells as the intact porphyrin complex. The heme oxygenase enzyme rapidly releases the iron. It mixes with other iron taken up by the cell before regulated transfer to the blood stream occurs. Heme iron is virtually always well absorbed (20-25%) and is little affected by other elements of the meal in which it is eaten. However, it amounts to only 10% or less of the dietary iron even in Western populations with a high meat consumption. The diets of most individuals in developing countries contain very little meat.

Common nonheme luminal pool. The rest of the dietary iron is in an inorganic form. This nonheme iron has a heterogenous origin, being derived from vegetable foods, inorganic contaminant iron, meat iron that is not in the form of heme, and inorganic iron fortificants added to the diet. The absorption of iron from all these sources requires prior solubilization. A common nonheme iron pool containing this soluble iron is formed in the lumen of the upper gastrointestinal tract. Absorptive mechanisms then extract iron from the pool. The amount of iron absorbed depends not only on mucosal behavior in the intestinal wall, but also on the presence of ligands in the meal, which either promote or depress iron absorption from the pool. Because nonheme iron absorption occurs before food has been exposed to the most powerful digestive enzymes, it is not surprising that the ligands present in undigested or partially digested foods play a major role in absorption. Several of these enhancers and inhibitors of nonheme iron absorption have been identified (Table 2). Their central role in modifying the percentage absorption of all nonheme iron in a meal (including any added fortification iron) is well established.

Insoluble iron. A variable proportion of iron ingested is in forms that are not readily solubilized in the upper gastrointestinal tract. This iron does not enter the common nonheme pool and is essentially unavailable for absorption. Such iron may contami-

Table 2 Enhancers and Inhibitors of Nonheme Iron Absorption

Absorption affecters	Food source
Enhancers	
Ascorbic acid	Fruits and vegetables
Animal tissue	Meat, fish, poultry
Inhibitors	
Polyphenols	Tea, coffee, some vegetables (e.g., eggplant, spinach), sorghum, lentils
Phytate	Cereals (e.g., wheat, maize, rice), legumes (e.g., soybean products)
Calcium	Milk
Protein	Soybean protein products, milk, manufactured foods containing bovine casein

nate meals as a result of primitive methods of preparation and storage; may occur naturally in certain foods, such as intact rice grains; or may be added to the food as an insoluble iron fortificant.

Strategies for Controlling Iron Deficiency

The public health importance of nutritional anemia has been recognized by the World Health Organization (WHO) since 1949 (Baker and DeMaeyer, 1979). WHO and other international and national agencies have sponsored efforts directed towards understanding factors affecting iron balance and designing methods for eliminating iron deficiency anemia. In general, strategies have been based on one or more of the following elements:

- *Iron supplementation*—providing medicinal iron usually as an oral preparation but occasionally as an injection.
- *Iron fortification*—adding iron to regularly consumed foods in the diet with or without absorption enhancers.
- *Reduction of iron loss*—for example, treating or preventing hookworm infestation.

Iron supplementation. This has the potential for producing a rapid improvement in iron status. It is particularly useful when a small segment of a population with the greatest need can be identified. The method is also most suited to situations where iron requirements are increased for a predictable and short period of time only (e.g., during pregnancy). Unfortunately, its effectiveness is often limited by the occurrence of gastrointestinal side effects, the difficulty of both instilling and sustaining the motivation required for adequate compliance, and the necessity of having an effective

health care delivery system to distribute or administer the iron preparation.

For situations where a chronic imbalance between iron supply and iron requirements exists, iron supplementation, which should only be given for short periods of time, is only a temporary solution. If increased requirements continue after the supplementation period, iron balance can again only be achieved by increased absorption from the diet. Moreover, if supplementation provided iron sufficient to restore body stores, these stores will be used first; iron absorption increases only when body iron stores fall. The percentage absorbed is proportional to the size of the remaining iron store and will be maximal only when the iron store is again exhausted. When dietary bioavailability is low, negative iron balance may persist even when the physiologic adaptive mechanisms are again maximized, and anemia will recur.

The speed with which iron deficiency recurs is a function of the individual's iron requirement and the size of the iron store achieved at the end of the supplementation period. If demands are low, iron stores are depleted very slowly. By way of illustration, Cook (1990) provided a hypothetical example. He calculated that a normal adult male with 1000 mg iron stores and a normal daily requirement of 1 mg would take 3-5 years to deplete this store even if his iron supply were totally eliminated (i.e., his diet contained no iron). By comparison a woman of childbearing age would be unlikely to attain an iron store exceeding 300 mg. If high menstrual losses increase her daily iron requirement to 1.5–2.5 mg and she is deprived of an oral iron supply, her iron store will be depleted in only 6-12 months. It is therefore evident that individuals with high iron requirements, whether it be for rapid growth in childhood or to meet the needs of menstrual loss and pregnancy in adult women, must have a continuous source of highly bioavailable iron in the diet. Because of the relatively small amount of heme iron in all human diets and its virtual absence from meals eaten in developing countries, the amount of bioavailable iron in the nonheme pool becomes the determining factor in controlling iron status.

Iron fortification. Evaluating the information on nutritional iron deficiency gathered over the last 40 years has led most investigators to conclude that iron fortification of the diet is the only cost-effective long-term strategy for reducing the prevalence of iron deficiency in most developing countries (Cook and Reusser, 1983; MacPhail and Bothwell, 1992). Food has been fortified with vital nutrients including iron in several Western countries, including the United States, Canada, Sweden, and the United Kingdom, since the early 1940s. Other countries that require wheat flour to be fortified with iron include Chile, Denmark, Guiana, Kenya, Zambia, and Nigeria (Barrett and Ranum, 1985). In the United States, guidelines have also been established for fortifying corn and rice.

The standards set by most countries for fortifying wheat flour, bread, and pasta products are based on restoring the quantity of iron removed by milling. Other foods, such as infant formulas and ready-to-eat breakfast cereals in the United States, have been used as vehicles to deliver additional iron (i.e., an amount greater than that found in the raw material). The aim has been to reduce further the prevalence of iron deficiency.

The prevalence of iron deficiency in most Western countries has declined remarkably since iron fortification was first introduced. An analysis of information gathered in the United States between 1976 and 1980 indicates that less than 6% of the most vulnerable groups (pregnant women and young children) suffer from iron deficiency anemia (Dallman et al., 1984).

There is little doubt that iron fortification plays an important role in ensuring adequate iron nutrition in infants who are not breast-fed in the United States (Dallman, 1990). Most pediatricians consider that iron-supplemented formula provides a reliable source of iron during the first year of life (Committee on Infant Nutrition, 1976). Infant cereal products are usually also fortified.

It is less certain that the introduction of iron fortification is directly responsible for the improved iron status of older children and adults. Although there is a general impression that iron fortification has indeed had a favorable impact, the bioavailability of some of the iron compounds used for fortification has been questioned (Elwood et al., 1971; Hallberg et al., 1986; Foman, 1987). In addition, several other factors have undoubtedly contributed to the improved iron status. They include the concurrent increase in affluence, a wider food selection, smaller families, the use of oral contraceptives, and self-supplementation with iron and vitamin preparations (Hallberg et al., 1979).

The efficacy of iron fortification in older children and adults is questionable because of the difficulty of adding iron to the diet in a readily absorbed form. The choice of a suitable iron source for dietary fortification has presented legislators, grain product suppliers, and the food industry with a major dilemma. Forms of iron that are easily added to food without causing changes in color, taste, or appearance are generally poorly absorbed and therefore very unlikely to improve iron nutrition. On the other hand, highly bioavailable forms of iron, such as ferrous sulfate, may affect the storage properties and the acceptability to the consumer of the final product (Barrett and Ranum, 1985). For these reasons compromises have been made. An iron source has sometimes been selected solely for its stability and lack of interaction with other components in the food. Fortification with this type of compound is usually futile. Frequently a balance has been struck between the bioavailability of a compound and its lack of reactivity with the vehicle. The use of a form of elemental iron is a good example of such a compromise. However, a Swedish study (Hallberg et al., 1986) suggests that the bioavailability of the carbonyl iron used in Sweden as a fortificant is much less than was reported in the initial absorption trials using elemental iron (Cook et al., 1973). Bioavailable iron compounds, such as ferrous sulfate, are the most likely to lead to improved iron balance. Unfortunately, their reactivity with other food elements restricts the conditions for using them.

The use of a bioavailable iron compound does not guarantee success. Fortification iron that is bioavailable enters the nonheme common pool before being absorbed. As outlined above, absorption from this pool is determined by the nature of the diet and depends on the balance between enhancers and inhibitors of iron absorption in the meal. Human absorption studies have demonstrated that even the addition of fairly

large quantities of bioavailable iron to strongly inhibitory foods is unlikely to improve iron balance significantly (Bothwell et al., 1979). Consequently considerable research has been devoted to identifying and attempting to modify the factors governing the bioavailability of iron in the common nonheme iron pool. Unfortunately, the direct application of much of this knowledge to the iron fortification of food is both expensive and difficult technically. The alternative approach of changing dietary habits is equally problematic because eating habits are deeply rooted in the cultures of most societies.

One way of improving iron bioavailability is to add ascorbic acid, which is a potent enhancer of iron absorption in the diet (Lynch and Cook, 1980). It can usually be added to foods without affecting consumer acceptability. Two factors have severely limited its usefulness. It is expensive compared with the cost of iron fortification compounds and it is unstable under uncontrolled storage conditions when exposed to oxygen and moisture. It is also unstable when food preparation involves baking or even prolonged boiling or reheating (Hallberg et al., 1982). Several methods of stabilizing ascorbic acid have been studied but have been either technically impractical or too expensive. The use of ascorbic acid to ensure bioavailability of common nonheme pool and fortification iron is only feasible for ready-to-eat foods that are produced and stored under carefully controlled conditions. Under these circumstances ascorbic acid plays a very valuable role, as has been well documented with infant formulas (Derman et al., 1980; INACG, 1986).

Implementation and evaluation of iron fortification. The only studies using modern methods for evaluating iron status that have demonstrated clearly that a well-designed iron fortification strategy can significantly reduce the prevalence of iron deficiency in the population come from developing countries. Several different methods have proven effective. They are all characterized by the use of an iron fortificant that had been demonstrated to be highly bioavailable under field conditions. The first example comes from Chile. In 1972 a field trial was carried out in which milk fortified with iron (15 mg/L as ferrous sulfate) was fed to infants (INACG, 1986). Although some benefit was seen, iron deficiency remained prevalent at the end of the trial. The authors concluded that the limited response was due to the poor bioavailability of the iron in the fortified milk. A second field trial was therefore undertaken in 1976.

Full-fat milk fortified with the same amount of ferrous sulfate was again used, but ascorbic acid (100 mg/L) was also added. Both the pilot studies and the subsequent regional field trial demonstrated a marked reduction in the prevalence of iron deficiency. Note that the results of this trial, together with a considerable body of supporting evidence from human absorption studies, suggest that adequate bioavailability of iron in infant foods can only be ensured if the iron salt is given with adequate quantities of ascorbic acid (Derman et al., 1980).

Another fortification strategy for which claims of improved hemoglobin concentrations and a reduced prevalence of anemia have been made is one in which table salt was fortified with ferrous sulfate. The ferrous sulfate was stabilized with a combina-

tion of orthophosphoric acid and sodium acid sulfate (Working Group on Fortification of Salt with Iron, 1982). The color, taste, and storage properties of the salt were reported to be good, and the use of the fortified salt proved efficacious in a multicenter trial. Although potentially applicable in certain situations, this approach is limited by the lack of central distribution points for salt in many countries.

The third strategy involves the use of sodium iron ethylenediaminetetraacetic acid (NaFeEDTA). This compound is particularly attractive because of its chemical stability and the flexibility of its application as a food fortificant. The iron-EDTA complex is relatively stable under most storage conditions. The absorption of iron from the complex has been studied in humans for almost 40 years and has repeatedly been shown to be less affected than other forms of iron by inhibitors in vegetable diets. This large body of evidence makes predictions about iron absorption from meals containing EDTA much more reliable than is the case for most other iron compounds. Moreover, three successful fortification trials using NaFeEDTA have been reported to date (*see* Chapter IV). All three studies provide convincing evidence that NaFeEDTA can be an effective iron fortificant even under conditions in developing countries that do not favor iron absorption. Although the choice of the fortification vehicle in each of these studies was a condiment, there is good evidence to support the claim that NaFeEDTA would be equally effective if added to many other food items, including dietary staples such as wheat flour and other cereals.

Conclusions

Despite the continuing high prevalence of iron deficiency in many developing countries and 40 years of research, iron fortification is not practiced in developing countries. There are many reasons for this, but one of the most important is the practical difficulty of adding bioavailable iron to food in a form that will ensure its absorption. It is against this background that NaFeEDTA merits further consideration. It has as yet not been used because there has been some concern about the safety of prolonged ingestion of EDTA and of possible interactions with other trace metals. Although other EDTA chelates have been approved as food additives and used for many years, NaFeEDTA was just recently provisionally considered by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to be safe in food fortification programs in populations in which iron deficiency anemia is endemic (JECFA report, 1993). A commercial supply of food-grade material is not currently available, but methods for its manufacture are well established and agricultural-grade material is widely used. The JECFA requested that specifications on assay and purity data for food-grade material and on analytical methodology be developed.

Faced with the realities of the extraordinary difficulty of designing a method for cost-effective food fortification in developing countries, we believe that NaFeEDTA has certain unique advantages and may be an extremely useful fortificant under these conditions. Unfortunately, much of the information regarding NaFeEDTA is dispersed in the literature. Therefore, the aims of this monograph are to:

- review information available on the chemical properties, physiologic effects, action in promoting iron absorption, and potential value as a fortificant of NaFeEDTA;
- evaluate critically published information on the toxicology of EDTA and its metal complexes; and
- identify the most appropriate potential applications for NaFeEDTA as an iron fortificant.

II. Chemical Properties and Physiologic Effects of EDTA-Metal Complexes

EDTA is a hexadentate chelator capable of combining stoichiometrically with virtually every metal in the periodic table (Chaberek and Martell, 1959). The metal ions are bound via four carboxylate or two tertiary amine groups. With divalent or trivalent metal ions a neutral or anionic metal chelate is formed. As a result the metal is largely prevented from reacting with competing anions and its solubility is greatly increased. The effectiveness of EDTA as a chelator for a particular metal ion depends on its stability constant with the metal ion. This is affected by pH, the molar ratio of chelator to metal ion, and the presence of competing metal ions capable of forming complexes with EDTA (Plumb et al., 1950; Martel, 1960; Hart, 1984). The stability constants for different EDTA-metal complexes vary considerably 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 Cu^{2+} with 18.4, Zn^{2+} with 16.1, Fe^{2+} with 14.6, Ca^{2+} with 10.6, Mg^{2+} with 8.7, and Na^{+} with 1.7 (West and Sykes, 1960). The situation is somewhat complicated by each metal having an optimum pH for chelate formation ranging from pH 1 for Fe^{3+} to pH 3 for Cu^{2+}, pH 4 for Zn^{2+}, pH 5 for Fe^{2+}, pH 7.5 for Ca^{2+}, and pH 10 for Mg^{2+} (West and Sykes, 1960). When NaFeEDTA is ingested with foods, the Fe^{3+} ion is expected to remain firmly bound to the EDTA moiety during passage through the gastric juice but could be exchanged for Cu^{2+}, Zn^{2+}, Fe^{2+}, or Ca^{2+} in the duodenum. Similarly when Na_2EDTA and $CaNa_2EDTA$ are consumed with foods, the Na^{+} and Ca^{2+} ions could be exchanged in the gastric juice for Fe^{3+} ions, which could in turn be exchanged for Cu^{2+}, Zn^{2+}, Fe^{2+}, or Ca^{2+} further down the gastrointestinal tract. The extent to which the various EDTA-metal complexes form is dependent on the pH and the concentration of the competing metals as well as other competing ligands. The lower stability constant and higher pH optimum of the Mg-EDTA chelate make reaction with Mg^{2+} less likely.

Iron and EDTA Absorption from NaFeEDTA

An appreciation of the chelating properties of EDTA with respect to iron provides the basis of our understanding of the observed effects of EDTA on food iron absorption. Ferric food iron is poorly absorbed by humans because it is precipitated from solutions with pH above 3.5 unless suitable complexing agents are present. It may therefore be partially insoluble in the upper small intestine, where most nonheme

iron is absorbed (Conrad and Schade, 1968; MacPhail et al., 1981). When EDTA i present in a meal, iron (primarily Fe^{3+}) remains in a complex with EDTA under acidi conditions in the stomach. The chelate holds the iron in solution as the pH rises in th upper small intestine, but the strength of the complex is progressively reduced allowing at least partial exchange with other metals and the release of some of the iror for absorption.

There is convincing evidence that iron chelated by EDTA (NaFeEDTA) is availabl for absorption via the physiologically regulated pathways responsible for iron uptak and that before absorption, iron is dissociated from the EDTA moiety (Candela et al., 1984). Studies in swine using a dual-labeled $Na^{59}Fe[2\text{-}^{14}C]EDTA$ preparation demonstrated rapid transfer of ^{59}Fe to the plasma with a peak at 1 hour and subsequent incorporation of 4.6% of the administered dose into circulating hemoglobin. A small fraction (0.3%) of the ^{59}Fe administered was excreted in the urine. In contrast to the ^{59}Fe, only a small percentage of the ^{14}C could be detected in the plasma at any time. Absorption occurred over an extended period (5-20 hours). A total of about 5% of the ^{14}C-labeled EDTA was eventually absorbed in the duodenum and jejunum and quantitatively excreted in the urine. In a parallel experiment, 5 mg iron as $Na^{59}FeEDTA$ was given to six fasting human volunteers. The mean radioiron absorption as measured by red blood cell utilization was 12.0%. Only 0.3% of the administered dose of iron was excreted in the urine. These studies demonstrate that iron and EDTA are absorbed independently when NaFeEDTA is administered by mouth.

Similar conclusions were reached in an earlier human absorption study carried out by MacPhail et al., (1981). $Na^{59}FeEDTA$ was administered to human volunteers. Between 3% and 25% of the ^{59}Fe was absorbed, but less than 1% of the administered ^{59}Fe appeared in the urine over the subsequent 24 hours. These findings again demonstrate that most of the iron must have been released from the EDTA complex before absorption, because ^{59}Fe absorbed in the form of the intact $Na^{59}FeEDTA$ complex would be expected to be excreted in the urine within 24 hours (*see* Metabolism of EDTA-Metal Complexes). Similar conclusions have been reached with another iron chelator (nitrilotriacetic acid), the properties of which have been studied extensively in experimental animals (Simpson and Peters, 1984).

In summary, most iron absorbed after NaFeEDTA is ingested is released to the physiological mucosal uptake system before absorption. Only a very small fraction of the NaFeEDTA complex (less than 1%) is absorbed intact, and it is completely excreted in the urine. An additional small fraction (probably less than 5%) of the EDTA moiety itself is absorbed, presumably bound to other metals in the gastrointestinal tract, and it is also completely excreted in the urine (Figure 1).

The measurement of iron absorption from NaFeEDTA does not reflect EDTA absorption from NaFeEDTA. The absorption of the EDTA moiety from EDTA-metal complexes has been studied by using ^{14}C-labeled EDTA. When ^{14}C-labeled $CaNa_2EDTA$ was fed to human subjects, less than 5% of the EDTA was absorbed, with the absorbed fraction being excreted quantitatively in the urine (Foreman et al., 1954). Similar results were obtained with a tightly bound chelate, $^{51}CrEDTA$, from which any released metal is very poorly absorbed (Bjarnason et al., 1983; Aabakken and Osnes,

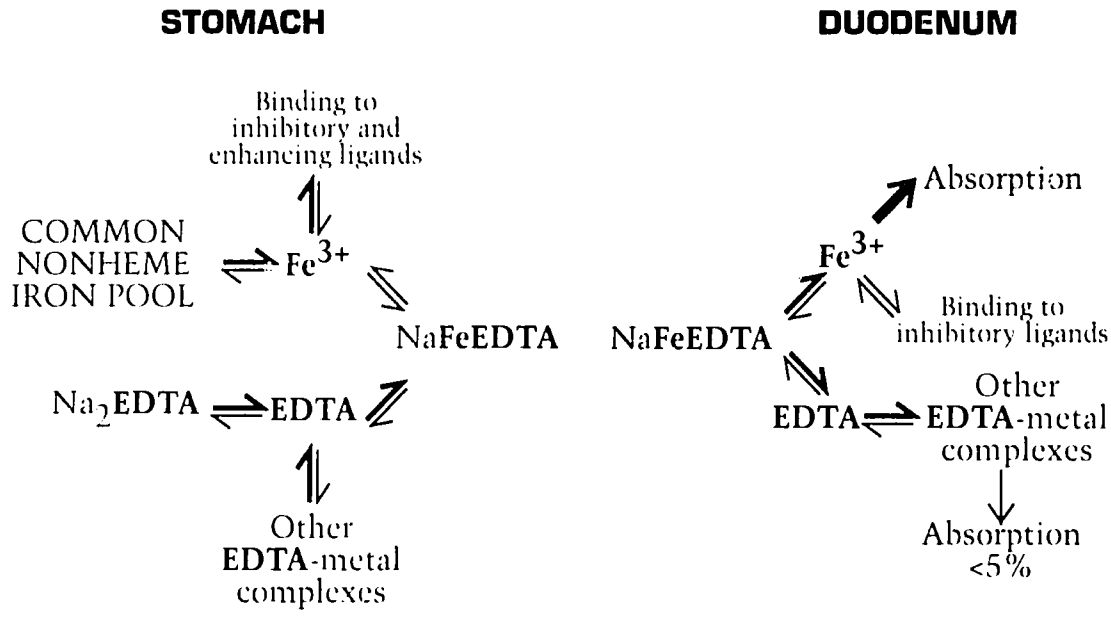

Figure 1

Interaction between EDTA and metal ions in the lumen of the stomach and duodenum.

1990). Only 1-5% of a dose of ^{51}CrEDTA given to a fasting subject is absorbed by the healthy intestinal mucosa. Essentially all absorbed ^{51}CrEDTA is excreted in the urine. In the presence of disorders of the gastrointestinal tract the absorption may be doubled. The absorbed ^{51}CrEDTA appears to be taken up through intercellular junctions as the intact complex. The amount absorbed (determined by quantitating urinary ^{51}Cr after the oral administration of ^{51}CrEDTA) has been used as a measure of the integrity of the bowel mucosa.

Although the absorption of the EDTA moiety from administered NaFeEDTA has not been measured directly in humans, physicochemical considerations indicate that EDTA absorption from NaFeEDTA would be similar to that from other metal complexes, such as $CaNa_2EDTA$ and CrEDTA. As described above, poor absorption of the intact NaFeEDTA can be inferred from measurements of urinary radioiron excretion after the oral administration of Na^{59}FeEDTA (MacPhail et al., 1981).

Metabolism of EDTA-Metal Complexes

The metabolism and organ distribution of [^{14}C]EDTA after intravenous administration of $CaNa_2$[^{14}C]EDTA has been studied both in experimental animals and in human volunteers. Studies in rats demonstrated that 95–98% of the EDTA was excreted in the urine within 6 hours. The chelate was not concentrated in organs and less than

0.1% of the material was oxidized and expired as $^{14}CO_2$ (Foreman et al., 1953). Similar results were obtained in humans (Foreman and Trujillo, 1954). Almost all of the chelate could be accounted for in the urine within 24 hours, 50% being excreted within 1 hour and 90% within 7 hours.

NaFeEDTA injected intravenously into rats shares the properties of other EDTA-metal chelates. Most of the iron (70-90%) is lost through the urine within 24 hours (Najarajan et al., 1964; Anghileri, 1967). A small proportion of the iron enters the physiological iron pool . . . which is destined primarily for hemoglobin synthesis. Its entry into this pool probably results from the slow release of iron from EDTA to transferrin, the iron-transport protein in the circulation (Bates et al., 1967). After intramuscular or intraperitoneal injection a greater proportion of the iron is available for physiological exchange with compartments in the bone marrow and liver. The longer contact time between transferrin and EDTA allows for greater transfer of iron from the chelate to the transferrin (Rubin et al., 1970).

There has been concern that small amounts of EDTA compounds consumed over a long period of time might negatively affect the nutritional status of zinc, copper, calcium, and possibly magnesium by the formation of nonmetabolizable metal-EDTA complexes. These complexes could be formed in the gastrointestinal tract and excreted directly in the stool or absorbed from the gut and excreted unchanged in the urine. EDTA could also be absorbed with one metal and excreted with another. The degree to which the exchange of iron bound to EDTA for other metals might occur would depend on the relative quantities of the different metals in a normal diet, the amount of EDTA present, and the stability constants of the different EDTA-metal chelates.

Food fortification with NaFeEDTA could be expected to influence zinc and copper metabolism but not calcium and magnesium metabolism. The Recommended Dietary Allowances (RDAs) of calcium, magnesium, and zinc for adult men (NRC, 1989) are 800 mg, 350 mg, and 15 mg respectively. No RDA was given for copper, but a daily intake of 1.5–3 mg was considered adequate and safe. A diet containing these amounts of calcium, magnesium and zinc and 2 mg/day of copper that was fortified with 10 mg iron as NaFeEDTA would contain equimolar quantities of EDTA and zinc, an 8-fold molar excess of EDTA over copper, but 80-fold less EDTA than calcium and 50-fold less EDTA than magnesium on a molar basis. The small quantity of chelator with respect to calcium and magnesium would be unlikely to have any effect. On the other hand, contrary to the expectation of some workers in the field, both NaFeEDTA and Na_2EDTA may increase the absorption and retention of zinc and copper when added to low-bioavailability diets. Animal studies support this conclusion (Forbes, 1961). Experiments with turkey poults (Kratzer et al., 1959), chicks (Scott and Ziegler, 1963), and rats (Forbes, 1961) demonstrated that zinc bioavailability and animal growth are improved when Na_2EDTA is added at 150-300 mg/kg to animal rations based on soybean protein isolate.

The enhancing effect of EDTA on zinc absorption in these studies is due to a combination of two factors. First, EDTA forms soluble chelates with zinc from which the metal is potentially absorbable, and second, zinc is prevented from forming

nonabsorbable complexes with phytic acid. EDTA does not enhance zinc absorption when absorption inhibitors are absent from a meal. Na_2EDTA (1000 mg/kg) improved zinc absorption by rats fed a casein-based diet with added phytic acid but had no effect in the absence of phytic acid (Oberleas et al., 1966). Other chelating substances can also enhance zinc absorption from low-bioavailability diets. Vohra and Kratzer (1964) compared the growth-promoting effect of chelates with stability constants for zinc (log k) varying from 5.3 to 18.8 in turkey poults fed zinc-deficient diets based on soy protein isolate. They found that ethylenediaminediacetic acid-dipropionic acid, hydroxyethyl-EDTA, and EDTA (stability constants 14.5, 14.5, and 16.1, respectively) were the most effective.

These earlier observations were made with Na_2EDTA. However, NaFeEDTA was shown to have similar properties in a recent study (Hurrell et al., 1993). Zinc, copper, and calcium balance studies were performed in rats fed low-zinc (6.1 mg/kg) soybean-based diets containing 50 mg iron/kg, of which 36 mg/kg was added iron as either ferrous sulphate or NaFeEDTA. In some experimental groups, additional Na_2EDTA was added to the diet containing NaFeEDTA to give dietary EDTA concentrations of 200, 500, and 1000 mg/kg (Table 3). Merely changing the iron compound in the diet from ferrous sulphate to NaFeEDTA increased apparent zinc absorption, urinary zinc excretion, and zinc retention significantly ($P < 0.05$) but caused no changes in copper and calcium absorption or excretion. The EDTA:zinc molar ratio in this study was 7:1. Increasing the dietary EDTA concentration to 500 mg/kg (EDTA:zinc molar ratio 19:1) and 1000 mg/kg (EDTA:zinc molar ratio 37:1) further increased both zinc

Table 3 Influence of NaFeEDTA and Increasing Concentrations of Na_2EDTA on 4-Day Zinc, Copper, and Calcium Balances in Rats

Diet and dietary EDTA (mg/kg)	Apparent absorption (% of intake)			Urinary excretion (% of intake)			Apparent retention (% of intake)		
	Zinc	Copper	Calcium	Zinc	Copper	Calcium	Zinc	Copper	Calcium
A, 0	50.2^a	27.5^a	57.1^a	2.0^a	3.9^a	1.2^a	48.2^a	23.6^a	55.9^a
B, 200	67.4^b	32.1^{ab}	53.3^a	4.0^b	3.8^a	1.1^a	63.4^b	28.3^{ab}	52.2^a
C, 500	79.4^c	38.2^b	55.3^a	6.7^c	4.0^a	1.8^{ab}	72.7^c	34.2^b	53.5^a
D, 1000	78.1^c	31.1^{ab}	57.9^a	15.6^d	4.1^a	2.3^b	62.5^b	27.0^{ab}	55.4^a

Diets contained 6.1 mg zinc, 9.7 mg copper, and 4.95 g calcium per kg.

The dietary iron compound was ferrous sulphate in diet A and NaFeEDTA in diets B, C, and D. Diets C and D also contained, respectively, 300 and 800 mg/kg of EDTA added as Na_2EDTA.

Each value is a mean of 8 observations. Means with different superscripts are significantly different ($P < 0.05$).

Source: Hurrell et al. (1993).

absorption and urinary zinc excretion. At the highest dietary EDTA concentration (1000 mg/kg), zinc retention was significantly higher than that with no dietary EDTA but lower than that with 500 mg/kg EDTA. This resulted from an increase in urinary excretion of zinc to 15.6% of intake. Similar results were obtained with a zinc-sufficient (30 mg/kg) soybean diet, but more EDTA was required to achieve optimal ratios for improved absorption. Thus neither absorption nor retention of zinc was increased with the addition of 500 mg EDTA/kg diet (EDTA:zinc molar ratio 3.7:1). The addition of 1000 mg EDTA/kg diet (EDTA:zinc molar ratio 7:5:1) did increase zinc absorption and retention.

Copper absorption and retention were improved at 500 mg EDTA/kg (EDTA:copper molar ratio 11.2:1) but not at 200 mg (EDTA:copper molar ratio 4.5:1) or 1000 mg EDTA/kg diet (EDTA:copper molar ratio 22.4:1). Apart from a very small increase in urinary calcium excretion, dietary EDTA had no influence on calcium metabolism.

For all the metals tested there appears to be a minimum EDTA:metal molar ratio below which absorption is not increased and an optimum ratio above which no further improvement in metal absorption and retention is seen. At very high concentrations of EDTA, metal absorption may actually diminish. This has been reported for zinc (Suso and Edwards, 1968) and iron (Cook and Monsen, 1976). When Swenerton and Hurley (1971) fed pregnant rats diets containing a 58-fold molar excess of EDTA over zinc, fetuses were malformed because of a zinc deficiency induced by EDTA.

A human diet containing the RDA of zinc (10 mg) and 2 mg copper and fortified with 10 mg iron as NaFeEDTA should contain equimolar quantities of EDTA and zinc and an eightfold molar excess of EDTA over copper. Therefore, on the basis of experimental observations in animals, NaFeEDTA fortification would be expected to have very little effect on zinc and copper balance. A small beneficial effect could occur in meals containing little zinc or copper or large quantities of phytate.

The applicability of observations made in experimental animals to human nutrition was confirmed recently (Davidsson, Kastenmayer, and Hurrell, unpublished data, 1992). The metabolism of zinc and calcium was studied by using a stable isotope technique in 10 adult women fed a breakfast meal of bread rolls made from 100 g high-extraction wheat flour fortified with 5 mg iron as ferrous sulphate or NaFeEDTA. The test meals contained a 3.3 molar excess of EDTA over zinc but some 10-fold less EDTA than calcium. Changing the iron fortification compound from ferrous sulphate to NaFeEDTA significantly increased ^{70}Zn absorption from the meal from 20.9% to 33.5% ($P < 0.05$). Urinary ^{70}Zn excretion also rose but only from 0.3% to 0.9%. Calcium metabolism was similar with the two different iron compounds.

Earlier human studies using less precise methodology reached similar conclusions about the effect of EDTA on zinc absorption. Solomons et al. (1979) reported that adding NaFeEDTA to a low-bioavailability Guatemalan meal did not influence zinc absorption by human subjects. However, because these workers determined zinc absorption by measuring plasma zinc concentrations after 25 mg zinc was ingested with a meal, the molar concentration of EDTA was some 10-fold less than that of zinc. An improvement in zinc absorption would not be expected. No significant changes in plasma zinc concentrations were observed in field studies in which NaFeEDTA was

used as a food fortificant over a 2-year period (Viteri et al., 1983; Ballot et al., 1989b).

In summary, the use of NaFeEDTA as a food fortificant would have no detrimental effect on the nutrition or metabolism of calcium, copper, zinc, or magnesium. In some situations, fortifying foods with NaFeEDTA could have a beneficial effect on zinc nutrition by improving zinc absorption.

Toxicology of EDTA Compounds

The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974) recommended that $CaNa_2EDTA$ or Na_2EDTA be permitted as food additives at a concentration providing up to 2.5 mg EDTA/kg body weight per day. These recommendations were based on the fact that $CaNa_2EDTA$ is poorly absorbed from the gut, is metabolically inert, and does not accumulate in the body. In addition, clinical experience with its use for treating metal poisoning demonstrated its safety in humans. A summary of the acute and chronic toxicity studies is given in the next sections.

Acute toxicity. The oral LD_{50} of $CaNa_2EDTA$ in rats, rabbits, and dogs was reported as 10, 7, and 12 g/kg body weight, respectively (Oser et al., 1963). The oral LD_{50} of Na_2EDTA was reported as 2-2.2 g/kg body weight for rats and 2.3 g/kg body weight for rabbits (JECFA, 1974).

Chronic toxicity. The long-term feeding study on which JECFA (1974) based its recommendation for the acceptable daily intake (ADI) of $CaNa_2EDTA$ was that of Oser et al. (1963). In this study, four groups of 25 male and 25 female rats were fed diets providing 0, 50, 125, or 250 mg $CaNa_2EDTA$/kg body weight per day for 2 years. At the highest amount, diets of rats older than 10 weeks contained 5g $CaNa_2EDTA$/kg of diet. The study included an investigation of reproduction through four successive generations.

No significant differences in body weight gain, efficiency of food utilization, blood sugar, serum nonprotein nitrogen, serum calcium, urine composition, organ weights, and histopathology of liver, kidney, spleen, heart, adrenals, thyroid, and gonads were observed. Fertility, lactation, and weaning were not adversely affected for each mating, and both mortality and tumor incidence were unrelated to dosage. Additional tests were made to investigate the possible influence of EDTA on mineral metabolism. There was no effect on the calcification of bones and teeth or on the activity of liver xanthine oxidase (a molybdenum-containing enzyme) or serum carbonic anhydrase (a zinc-containing enzyme).

At higher concentrations of $CaNa_2EDTA$ (10-40 g/kg diet), growth depression and anemia were observed. These effects were readily reversed by adding iron, copper, and other mineral salts to the diet. In a similar 2-year rat feeding study with Na_2EDTA, Yang (1964) reported no differences in weight gain, blood variables, bone ash, microscopic examination of various organs, and mortality at 0, 5, or 10 g Na_2EDTA/kg diet. At 50 g Na_2EDTA/kg diet, rats had diarrhea and consumed less food.

Oser et al. (1963) also reported on a feeding study in which four groups of 4-6-month-old mongrel dogs were fed diets providing 0, 50, 100, or 250 mg $CaNa_2EDTA$/kg body weight per day for 1 year. Every dog gained weight during the dietary period

regardless of the treatment. There were no differences in hematology, blood chemistry, urine composition, or histopathology of various organs, and there was no evidence of skeletal changes.

JECFA (1974) calculated the ADI of $CaNa_2EDTA$ from the rat study of Oser et al. (1963) by accepting the highest dose tested (250 mg/kg body weight per day) as the observed no-effect level and applying a safety factor of 100 to obtain an ADI in humans of 2.5 mg/kg body weight per day. If a higher dosage had been tested, the ADI could have been higher. JECFA (1974) also permits the use of Na_2EDTA in foods provided that no excess beyond that required for food processing or storage remains in the food when consumed. They recommended the use of $CaNa_2EDTA$ in preference to Na_2EDTA because Na_2EDTA can sequester calcium. This last statement appears questionable because of the chemistry of EDTA compounds outlined above.

Toxicological profile. Different endpoints have been evaluated in the toxicological evaluation of EDTA; including carcinogenicity, teratogenicity, genotoxicity, kidney damage, and hypersensitivity end points. The mechanism of toxicity central to most of these effects appears to be a depletion of metal ions.

There is no evidence that EDTA compounds are carcinogenic. In a study done for the United States National Cancer Institute (1977), Na_2EDTA was administered to rats and mice at 3.75 and 7.5 g/kg diet for 103 weeks. No compound-related signs of clinical toxicity were observed. Although a variety of tumors occurred in both test and control groups, no tumors were related to treatment.

At very high dietary concentrations, EDTA compounds ingested by pregnant rats were teratogenic. This appears to be due to zinc deficiency caused by the deleterious influence of very high concentrations of EDTA on zinc absorption and utilization. Swenerton and Hurley (1971) fed pregnant rats diets containing 20 or 30 g Na_2EDTA/kg diet and either 100 or 1000 mg zinc/kg diet. There was a high incidence of malformed fetuses in the rats fed diets containing 30 g Na_2EDTA/kg with 100 mg zinc/kg. An increase in zinc to 1000 mg/kg prevented the teratogenic effect. Kimmel (1977) found malformations in 71% of the offspring of pregnant rats fed diets containing about 25 mg zinc/kg with 30 g Na_2EDTA/kg (954 mg/kg body weight per day). A reduction in maternal food intake and growth was also observed. In contrast, Schardein et al. (1981) used gastric intubation to administer EDTA as Na_2EDTA, Na_3EDTA, Na_4EDTA, and $CaNa_2EDTA$ to female rats between days 7 and 14 of gestation. A dose equal to 1000 mg EDTA/kg body weight was used but no evidence of teratogenic effects was found. There was a high incidence of diarrhea in some groups. The dietary zinc concentration was not reported. However, the EDTA compounds were not fed with the food, and the dietary zinc absorption would therefore not be expected to be affected. On this basis, the absence of teratogenic effects is not surprising. In summary, the teratogenic effect of EDTA compounds has only been demonstrated in rats fed 1000 mg/kg body weight of the compound in the daily diet. This extremely high intake is 400-fold the ADI and appears to provoke teratogenicity by causing zinc deficiency, because the extremely high molar excess of EDTA with respect to zinc inhibits zinc absorption.

In recent years, EDTA has been tested for genotoxicity in assays measuring gene mutational and chromosomal effects. Dunkel et al. (1985) reported that Na_3EDTA did not increase the number of revertants in either *Salmonella typhimurium* or *Escherichia coli*. Moreover Na_3EDTA did not increase the mutant frequency in an in vitro mammalian cell forward-mutation assay (McGregor et al., 1988). In studies sponsored by the United States National Toxicology Program (unpublished data), Na_3EDTA did not induce chromosomal aberrations or sister chromatid exchanges in mammalian cells in culture. On the basis of these results, it is unlikely that EDTA is a harmful genotoxic chemical.

Raymond and Gross (1969) reported a 6% (3/50) positive response to $CaNa_2EDTA$ with two of the three positively responding individuals cross-reacting with ethylenediamine in an early human skin-sensitization study. However, 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. From their negative results, they concluded there was an extremely low potential for sodium salts of EDTA to produce a sensitization response in human skin.

Nephrotoxicity is the main hazard associated with the parenteral administration of $CaNa_2EDTA$ or Na_3EDTA for treating metal poisoning in humans, and there are many reports of both toxicity and kidney damage associated with overdosage. (Holland et al., 1953; Dudley et al., 1955; Reuber and Bradley, 1960). Renal lesions were observed in all cases. The proximal convoluted tubules revealed changes ranging in severity from vacuolization of the epithelium to an acute necrotizing nephrosis. Toxicity studies in rats showed microscopic changes restricted almost exclusively to the kidney, with lesions similar to those observed in humans (Foreman et al., 1956; Reuber and Schmieler, 1962). Unlike $CaNa_2EDTA$, the more stable chelate CrEDTA was nontoxic when infused intravenously into dogs (Ahrens and Aronson, 1971), indicating that the toxicity of EDTA is due to its chelating properties rather than to some nonspecific action (Ahrens and Aronson, 1971).

Potential toxicity of NaFeEDTA. From the chemical properties of EDTA complexes, it can be predicted that the EDTA moiety of NaFeEDTA consumed with a food behaves physiologically similarly to the EDTA moiety from $CaNa_2EDTA$ or Na_3EDTA. The same toxicological considerations should therefore apply. The argument on which we base these conclusions has been given in an earlier section of this chapter and is only summarized here. Regardless of whether the EDTA moiety is consumed as $CaNa_2EDTA$, Na_3EDTA, or NaFeEDTA, once in the digestive tract it forms complexes in the same way and has the same metabolic fate. NaFeEDTA is formed in the stomach where the pH is low. As the NaFeEDTA complex passes down the intestinal tract, the luminal pH rises because of the inflow of bile and pancreatic secretions. The iron is displaced from the EDTA complex by other metals, the main ones probably being zinc and copper. A variable amount of the iron is absorbed independently of the EDTA moiety. A small fraction (approximately 5%) of the EDTA moiety is also absorbed, but most of this forms complexes with a metal or metals

other than iron regardless of the EDTA compound originally consumed. Less than 1% of the iron is absorbed as the intact NaFeEDTA complex. Thus the results of acute and chronic toxicity studies and the toxicological profiles of Na_2EDTA and $CaNa_2EDTA$ derived from experiments in which the EDTA compounds were administered with food apply equally to NaFeEDTA. The most significant potential consequence of the prolonged administration of large quantities of an EDTA complex is zinc deficiency. However, EDTA in the concentration range that would be present in the diet as a result of food fortification with NaFeEDTA would either not affect zinc balance or under some circumstances actually improve it.

Current Use of EDTA Compounds and Regulatory Issues

On the basis of JECFA (1974) recommendations, many countries in Europe, Asia, North and South America, Africa, and Australia have permitted the addition of Na_2EDTA and $CaNa_2EDTA$ to foods. Both compounds are presently added to a wide range of foodstuffs as sequestering agents to prevent off flavor, discoloration, turbidity, rancidity, and texture loss. They are used most often in mayonnaise and other sauces, canned vegetables (peas, beans, and potatoes), canned fish and shellfish, carbonated beverages, beer, and margarine. Some countries have also permitted their use in mushrooms, brandy, glace cherries, spreads, banana products, dried and canned fruit, pickles, egg products, and sausages. Occasionally they are permitted to be added to meat extracts, soups, jams, soybean milk, ketchup, milk products, cocoa, chocolate, pasta, bread, breakfast cereals, cheese, ice cream, and confectionery. Very small amounts of EDTA may also enter the food chain because of other approved uses in food processing and packaging materials.

The European Economic Community (EEC) has a fairly restrictive policy on the use of EDTA compounds. They are only permitted in canned crab (275 mg/kg), canned shrimp (250 mg/kg), pickles (220 mg/kg), canned mushrooms (200 mg/kg), glace cherries (100 mg/kg), and sauces (75 mg/kg). Their use is not allowed in foods destined for infants and young children. Other countries, such as the United States, Malaysia, and the Philippines, permit the addition of EDTA compounds to a much broader range of food products. The United States allows Na_2EDTA and $CaNa_2EDTA$ to be added to 34 different food products (Anonymous, 1991), some of which are listed in Table 4.

Estimated Daily Intake for EDTA in the United States

The United States Food and Drug Administration (FDA) has determined the estimated daily intake (EDI) for EDTA on several occasions since 1969. In 1992 the FDA updated the estimate of chronic exposure to EDTA by using available food intake information and Monte Carlo simulation methodology to calculate exposure. By this method the mean overall exposure to EDTA was estimated to be 15 mg/person per day (P. Whittaker et al., 1993). This updated EDI is substantially below the ADI of 150 mg/person per day and suggests that additional uses or forms of EDTA, such as the NaFe salt in selected foods, may be possible in the United States.

III. Iron Absorption from Meals Containing NaFeEDTA and Na_2EDTA in Humans

NaFeEDTA

As outlined in the introduction, choosing an iron compound for fortifying foods poses several important problems. Adding soluble bioavailable iron compounds to most foods produces unacceptable color changes. In addition, these forms of iron catalyze oxidative reactions, causing undesirable odors and flavors particularly if prolonged storage is required before the food is consumed. More-stable, less-soluble complexes tend to be poorly absorbed. As previously discussed, even when it is technically feasible to incorporate a bioavailable iron salt, the diet itself may profoundly affect the absorption of fortification iron. Diets in developing countries usually contain strong inhibitors of iron absorption whereas promoters of iron absorption, such as ascorbic acid and meat, are present only in limited amounts. (MacPhail and Bothwell, 1992). Fortification of these diets with soluble iron salts is negligibly beneficial because the fortificant iron and the intrinsic food iron are subjected to the same inhibitory factors. The iron chelate, NaFeEDTA, has three significant advantages in this dietary setting (MacPhail et al., 1985):

- FeEDTA is a pale yellow water-soluble powder that can be added unobtrusively to several food vehicles.
- When NaFeEDTA is eaten with foods containing large quantities of absorption inhibitors, the EDTA protects the iron from their effects, maintaining it in a bioavailable soluble form. As a result, iron supplied in the form of NaFeEDTA may be two to three times more bioavailable than $FeSO_4$ in some diets.
- NaFeEDTA is a stable compound even under adverse storage conditions. It is unaffected by cooking.

Iron absorption from NaFeEDTA-fortified foods. The results of iron absorption studies comparing the bioavailability of iron from foods fortified with ferrous sulphate and NaFeEDTA are listed in Table 5. For comparison, the individual absorption values have been standardized to a reference absorption of 40% to remove the influence of different iron requirements in different subjects. A reference absorption value of 40% is assumed to represent borderline iron deficiency (Hallberg et al., 1978). The bioavailability of iron from ferrous sulphate varied over a wide range and correlated

Table 5 Comparison of Iron Absorped from Meals of Different Iron Bioavailability Fortified with Ferrous Sulphate or NaFeEDTA

Meal components	Standardized iron absorption (%)[a] A Ferrous sulphate	B NaFeEDTA	Ratio B/A	Reference
Rice Milk	1.7	4.5	2.6	Viteri et al., 1978
Beans, maize, and coffee	2.0	5.3	2.7	Viteri et al., 1978
Egyptian flat bread[b]	2.1	5.3	2.5	el Guindi et al., 1988
Bran	2.7	7.8	2.9	MacPhail et al., 1981
Beans, plantain, rice, maize, and soy[c]	3.1	7.0	2.3	Layrisse and Martinez-Torres, 1977
Rice	3.9	11.5	2.9	A.P. MacPhail, unpub. 1992
Maize meal	4.0	8.2	2.1	MacPhail et al., 1981
Beans, plantain, rice, maize, soy, and meat[c]	4.2	7.4	1.4	Layrisse and Martinez-Torres, 1977
Beans, plantain, rice, maize, soy, and meat[c]	4.3	9.6	2.2	Layrisse and Martinez-Torres, 1977
Potato	5.9	7.3	1.2	Lamparelli et al., 1987
Wheat	6.2	14.6	2.3	Martinez-Torres, et al., 1979
Milk	10.2	16.8	1.6	Layrisse and Martinez-Torres, 1977
Sweet manioc	14.1	16.6	1.2	Martinez-Torres et al., 1979
Sugar cane syrup[c]	33.1	10.8	0.3	Martinez-Torres et al., 1979

[a]Geometric means standardized to a reference absorption of 40%.
[b]A mixture of ferrous sulphate and Na_2EDTA was used in this study.
[c]Comparison between ferrous sulphate and NaFeEDTA not in the same individuals.

with the relative proportions of enhancers and inhibitors known to be present in the meals. In contrast, the absorption of iron from NaFeEDTA eaten with identical meals varied only two- to threefold. With one exception, more iron was absorbed from the meals containing NaFeEDTA. Enhanced bioavailability was most marked in meals with poor ferrous sulphate bioavailability (ferrous sulphate absorption of less than 4%). Between 2.1 and 2.9 times as much iron was absorbed under such circumstances. This point is exemplified by the second study in Table 5 in which iron absorption from a NaFeEDTA-fortified meal of beans, maize, and coffee was 2.7 times greater than that from the same meal containing ferrous sulphate (Viteri et al., 1978). Significant amounts of both phytate and polyphenols were present in the meal.

The absorption of iron from NaFeEDTA has been studied in a wide variety of meals. Comparisons with iron absorption from simple iron salts have not always been made. However, these observations provide useful information about the suitability of several food items as potential vehicles for fortification with NaFeEDTA. The results obtained in 25 different studies are summarized in Table 6. Some of the studies listed in Table 5 have been included. It is evident that at least 10% of the fortification iron added would be absorbed from all but the most inhibitory meals by iron-deficient individuals.

Table 6 **Percentage Iron Absorbed from Meals Containing NaFeEDTA**

Food	Number of Studies	Mean absorption (range) (%)[a]	References
Maize	7	9.1 (7.9–12.0)	Martinez-Torres et al., 1979; MacPhail et al., 1981
Wheat	3	10.2 (5.3–14.6)	Martinez-Torres et al., 1979; el Guindi et al., 1988
Cassava	3	13.5 (11.0–16.4)	Martinez-Torres et al., 1979
Lentils	1	7.9	MacPhail et al., 1981
Rice	1	11.5	MacPhail et al., unpublished, 1992
Milk	1	16.7	Martinez-Torres et al., 1977
Mixed meal	9	7.2 (3.9–10.5)	Layrisse and Martinez-Torres, et al., 1977; Martinez-Torres et al., 1979; Lamparelli et al., 1987

[a]All values standardized to a reference absorption of 40% or serum ferritin of 26 μg/L.

Table 7 Absorption of Iron from NaFeEDTA and Intrinsic Food Iron in the Same Meal

Meal	Standardized absorption (%) A NaFeEDTA (^{59}Fe)	B Iron (^{55}Fe)	Ratio A/B	Reference
^{59}Fe maize porridge Na^{59}FeEDTA fortified sugar	12.9	4.9	2.6	MacPhail et al., 1981
^{55}Fe maize porridge Na^{59}FeEDTA cooked into meal	8.1	5.5	1.2	MacPhail et al., 1981
^{59}Fe dhal (lentils) cooked with Na^{59}FeEDTA	5.4	5.2	1.04	MacPhail et al., 1981
^{55}Fe maize in a mixed meal[a] Na^{59}FeEDTA added to dough	4.8	4.0	1.3	Layrisse and Martinez-Torres, 1977
^{59}Fe maize in a mixed meal[b] Na^{59}FeEDTA added to dough	6.2	5.1	1.2	Layrisse and Martinez-Torres, 1977
^{55}Fe wheat in a mixed meal[b] Na^{59}FeEDTA added to dough	9.6	8.6	1.1	Layrisse and Martinez-Torres, 1979

[a]Beans, plantain, rice, maize, soy, and meat.
[b]Beans, plantain, rice, maize, and soy.

Relationship of NaFeEDTA to the common nonheme iron pool. Conclusions drawn from much of the experimental work on food iron absorption and iron fortification are based on the observation that soluble iron added to a meal and the intrinsic nonheme food iron behave as a common pool (Cook et al., 1972; Hallberg and Bjorn-Rasmussen, 1972). Both sources of iron are equally susceptible to enhancers and inhibitors of iron absorption present in the meal. NaFeEDTA shares this property. When Na^{59}FeEDTA was added to meals labeled intrinsically with ^{59}Fe (Table 7) the ratio between the proportions of iron absorbed from the two sources was close to unity in all except the first study, which indicates that the Na^{59}FeEDTA must have equilibrated with the common pool (MacPhail et al., 1981). Inadequate mixing of the NaFeEDTA-fortified sugar with the maize meal probably accounted for the lack of equilibration.

These results reveal another important property of NaFeEDTA. Equilibration of NaFeEDTA with iron in the common pool improves the bioavailability of the intrinsic food iron as well. Therefore, NaFeEDTA improves iron balance in two ways: it supplies iron in a form less affected by dietary inhibitors and improves the absorption of nonheme iron in the meal derived from other sources. Figure 2 illustrates this point and demonstrates that the positive effects of EDTA on iron absorption are shared by other elements of the common pool, such as another iron salt added to the meal. In one experiment ferrous sulphate and NaFeEDTA were fed on separate days in the same type of meal (maize porridge). Iron absorption from the NaFeEDTA-fortified meal was found to be significantly better. However, the iron from ferrous sulphate was as well absorbed as that from NaFeEDTA when they were

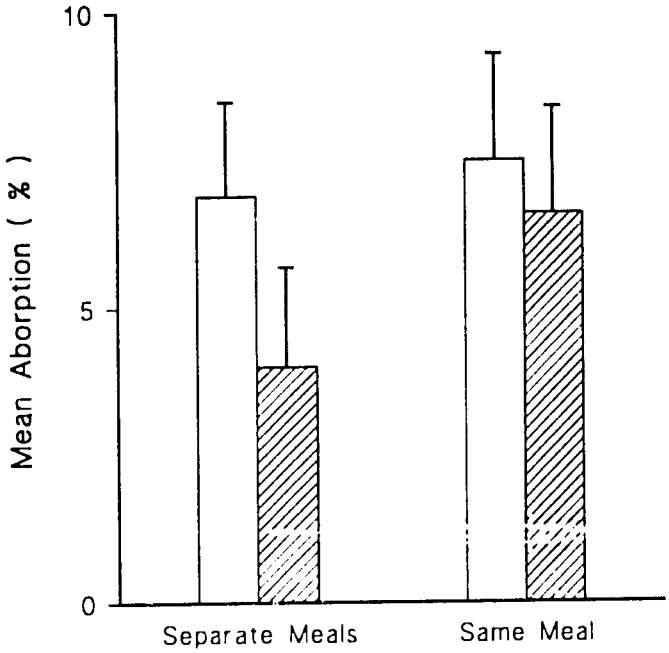

Figure 2

Mean absorption of iron (±1SD) from NaFeEDTA (open bars) and ferrous sulphate (hatched bars) when fed in separate meals and in the same meal. (Source: MacPhail et al., 1985, with permission of Academic Press.)

fed together in the same meal (MacPhail et al., 1981; Martinez-Torres et al., 1979). More direct evidence of reciprocal exchange between food iron and iron added as NaFeEDTA was provided by experiments in which subjects were given maize porridge fortified with equimolar quantities of $^{59}FeSO_4$ and $Na^{55}FeEDTA$. The ratio between the two isotopes was almost the same in the meal and the urine. This implies that exchange of iron between ferrous sulphate and NaFeEDTA must have occurred before absorption of the chelate, because only iron bound to EDTA is excreted in the urine (*see* Chapter II for explanation).

Effect of body iron stores on iron absorption from NaFeEDTA. There is a close inverse relationship between body iron stores and nonheme iron absorption. As body iron stores decrease, the percentage of iron absorbed from the nonheme common pool increases proportionally, becoming maximal when stores are completely exhausted. The use of the serum ferritin assay as a measure of the size of body iron stores has made it possible to define the relationship between body iron stores and iron absorption quantitatively (Bezwoda et al., 1979).

Changes in the size of iron stores have the same proportional effects on absorption from all meals regardless of the bioavailability of iron in a particular meal, and they provide the basis for correcting or standardizing absorption to the arbitrary ferritin concentration that represents storage iron depletion. Correction to a serum ferritin of 26 mg/L is equivalent to correction to a reference absorption of 40% (Baynes et al., 1987). The relationship is unchanged when NaFeEDTA is used as the iron source (J.D.

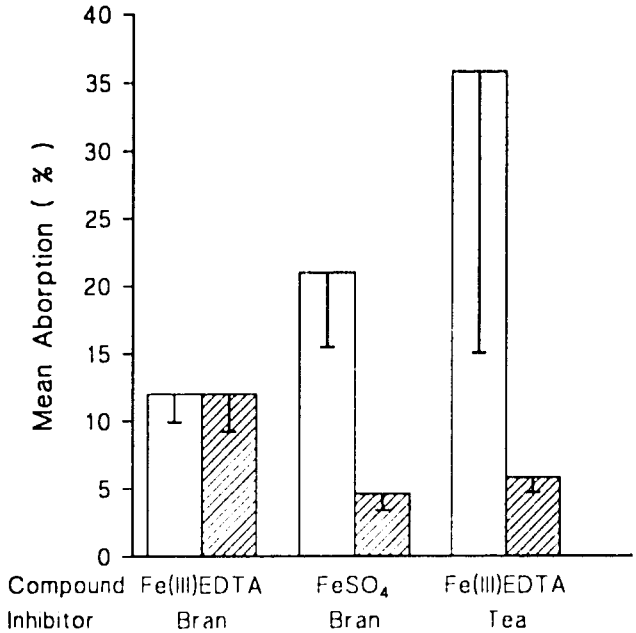

Figure 3

The effect of adding an inhibitor of iron absorption on the absorption of iron from aqueous solutions containing ferrous sulphate or NaFeEDTA. Values are geometric means and SDs. Open bars without inhibitor; hatched bars with inhibitor. (Source: MacPhail et al., 1985, with permission of Academic Press.)

Cook and S.R. Lynch, unpublished data, 1992). This finding provides further evidence of the availability of iron in NaFeEDTA to the physiologic absorptive mechanisms.

Effect of inhibitors and enhancers on iron absorption from NaFeEDTA. Phytate and polyphenols are among the most potent inhibitors of iron absorption (MacPhail and Bothwell, 1992). A few studies provide some information about the interaction between EDTA and specific dietary components. The effects on iron absorption of adding two powerful inhibitors, bran or tea, to aqueous solutions containing either ferrous sulphate or NaFeEDTA are shown in Figure 3. Phytate is the predominant inhibitor of iron absorption in bran and polyphenols (tannins) are the predominant inhibitor in tea. Bran reduces absorption from ferrous sulphate 11-fold. No such inhibition occurred when NaFeEDTA was present. The EDTA was capable of protecting the iron by holding it in a bioavailable form and preventing its bonding to phytate (Lyon, 1984; Hurrell et al., 1992). The polyphenols in tea are more potent inhibitors, and their presence was associated with a sevenfold reduction in the absorption of iron from NaFeEDTA. These findings indicate that polyphenols have a greater affinity for iron than does EDTA in the conditions existing in the upper gastrointestinal tract.

Ascorbic acid produces a dose-dependent enhancement of iron absorption from the common pool in the absence of EDTA (Sayers et al., 1973; Bjorn-Rassmussen and Hallberg, 1974; Derman et al., 1977). However, when NaFeEDTA and ascorbate were added together to a maize meal, iron absorption was enhanced only at the highest dose (Table 8). The limited effect of NaFeEDTA in diets of high bioavailability was also

Table 8 The Effect of Increasing Doses of a Promoter (Ascorbic Acid) on the Absorption of Iron (5 mg) from NaFeEDTA Added to Maize Porridge

Ascorbic acid (mg)	Ratio iron:ascorbic acid	Absorption (%)[a]	
		Without ascorbic acid	With ascorbic acid
25	1:1.5	8.5	8.9
50	1:3.0	12.0	12.0
100	1:6.0	8.0	15.6

[a]Geometric means standardized to a reference absorption of 40%.
Source: MacPhail et al. (1981).

observed in a meal containing meat. The nonheme iron absorption was the same regardless of whether iron was present as NaFeEDTA or ferrous sulphate (J.D. Cook and S.R. Lynch, unpublished data, 1992).

These experiments suggest that NaFeEDTA protects the iron in food from the inhibitory effects of phytate. Whether it also protects iron from the effects of other inhibitors is less certain. Its presence has no promoting effect in meals in which iron absorption enhancers predominate. Thus NaFeEDTA has no advantage in meals of high bioavailability and may sometimes even reduce iron absorption under such circumstances.

The Effect of Na_2EDTA on Iron Absorption

As previously discussed, Na_2EDTA is widely used as a food additive to prevent oxidative damage by free metals. Because Na_2EDTA readily chelates iron in the gut to form NaFeEDTA, its effect on iron absorption is of interest. Na_2EDTA and an equimolar quantity of iron as ferrous sulphate were added to bread with a high phytate concentration (82% extraction flour) (el Guindi et al., 1988). The combination was associated with a 2.6-fold enhancement in iron absorption when compared with results when ferrous sulphate was used alone. The mean percentage iron absorption was approximately equivalent to that reported in similar studies using NaFeEDTA (Table 5). The same effect on iron absorption can be achieved in inhibitory meals when Na_2EDTA and a soluble iron salt are added instead of NaFeEDTA.

The effects of Na_2EDTA on iron absorption appear to be influenced by the EDTA:Fe molar ratio. Earlier work suggested that increasing the Na_2EDTA:Fe molar ratio was associated with a progressive reduction in iron absorption (Cook and Monsen, 1976). These observations were extended recently (P.R. MacPhail and T.H. Bothwell, unpublished data, 1992). Iron absorption from a series of rice meals containing Na_2EDTA and in a 1:1 molar ratio was compared with rice containing Na_2EDTA and iron in molar ratios (EDTA:Fe) ranging from 1:4 and 1:1 (Figure 4). Statistically significant enhancement of absorption occurred at EDTA:Fe molar ratios between 1:4 and 1:1.

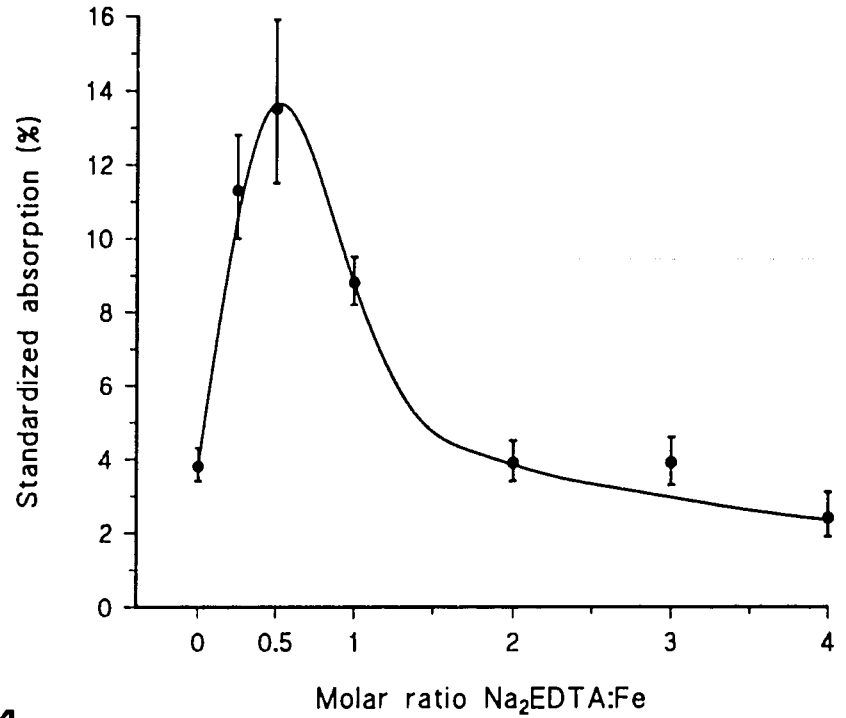

Figure 4

The effect of increasing amounts of Na_2EDTA on iron absorption from a rice meal fortified with $FeSO_4$ (total iron: 3 mg). Absorptions have been standardized to a serum ferritin of 26 µg/L, which is equivalent to a reference absorption of 40% (Baynes et al., 1987). Values are geometric means and SEs.

The enhancing effect of EDTA on iron absorption appeared to be maximal at an EDTA:Fe molar ratio of approximately 1:2 and not 1:1 as previously assumed. At this molar ratio more than three times as much iron was absorbed from the EDTA-containing meal as from the control meal without EDTA.

Conclusions

Absorption studies in humans indicate that the EDTA moiety in $Na_2FeEDTA$ protects iron from the effects of inhibitory ligands (especially phytate) in foods. When inhibitors are present, iron derived from NaFeEDTA is two to three times more available than it is from ferrous salts or food iron. Iron in NaFeEDTA exchanges with the nonheme iron in the diet and the EDTA moiety also enhances the bioavailability of the nonheme iron pool about twofold. The use of NaFeEDTA as an iron fortificant therefore holds promise for populations consuming diets of low-iron bioavailability. Its major application will be in developing countries where the diets are predominantly cereal based. In this setting an absorption of 8-10% of both the added fortification iron and the intrinsic food iron can be expected. Thus the daily intake of food fortified with 10 mg iron as NaFeEDTA would be expected to increase iron absorption in iron-deficient subjects by more than 0.8 mg/day.

The fortification with NaFeEDTA of diets rich in enhancers, such as those commonly eaten in the developed world, is less attractive (MacPhail and Bothwell, 1992).

Iron availability would not be improved and NaFeEDTA might even reduce the effects of other enhancers of iron absorption under some circumstances.

Consideration should also be given to the use of Na_2EDTA or $CaNa_2EDTA$ either alone or with another form of fortification iron. Preliminary findings suggest that an EDTA:Fe molar ratio of slightly less than 1:1 in diets of low-iron bioavailability could enhance iron absorption similarly to that seen with NaFeEDTA. The experimental evidence for this conclusion is only from studies using Na_2EDTA. $CaNa_2EDTA$ would be expected to have similar properties but has not been tested directly. In a rat study evaluating iron bioavailability, the same effect on iron absorption was achieved in an inhibitory meal when either Na_2EDTA and a soluble iron salt or NaFeEDTA alone was used (Whittaker and Vanderveen, 1990). With a less inhibitory meal, however, the combination of $FeSO_4$ and Na_2EDTA for fortification was significantly less effective than NaFeEDTA.

IV. Field Trials Using NaFeEDTA-Fortified Food

Fortification Strategy

The guidelines for developing a food fortification strategy were described in detail in previous INACG publications (INACG, 1977, 1984) and were further refined in subsequent reviews (Cook and Reusser, 1983; MacPhail and Bothwell, 1989; Bothwell and MacPhail, 1992). The stepwise approach to implementing a national program and the ideal characteristics for a food vehicle are summarized in Table 9. A good deal of the information relating to NaFeEDTA has already been collected. Perhaps the most difficult step is choosing a food vehicle (Table 10). It is unlikely that any vehicle will meet all listed criteria, but potentially suitable vehicles for NaFeEDTA can be selected on the basis of information presently available (*see* Recommendations).

As described earlier, NaFeEDTA has several advantages over other fortification compounds. It is a light color, relatively stable, and not affected by prolonged storage. Wheat flour containing NaFeEDTA has been stored for 1 year without any effect on its organoleptic properties (R.F. Hurrell, unpublished data, 1992). The iron bioavailability is satisfactory and unaffected by storage or cooking.

Field Trials Using NaFeEDTA

Three comprehensive field trials have been carried out using NaFeEDTA as an iron fortificant (Garby and Areekul, 1974; Viteri et al., 1983; Ballot et al., 1989b). The salient features of these trials are listed in Table 11; the guidelines (outlined in Table 9) were generally followed in each trial. All three trials were preceded by some estimate of the iron status of the population and care was taken to establish the acceptability and bioavailability of iron from the chosen vehicle before the trials

Table 9 Steps in Developing an Iron Fortification Strategy

1. Determine the iron status of the population.
2. Choose an appropriate iron compound and food vehicle combination.
3. Establish the acceptability and stability of the fortified vehicle.
4. Assess the bioavailability of iron from the vehicle in the appropriate dietary setting.
5. Carry out a controlled field trial.
6. Implement a regional or national fortification program.

Table 10 Considerations in Choosing a Food Vehicle for Iron Fortification

Consumption	Technical
High proportion of the population	Centrally processed
Minimal regional variation	Few production facilities
Unrelated to socioeconomic status	Minimal segregation of fortificant
Minimal individual variation	Good masking qualities
Low potential for excess intake	Low cost
Contained in all meals	Limited shelf life
Linked to caloric intake	High bioavailability

(Garby and Areekul, 1974; Viteri et al., 1983; Lamparelli et al., 1987; Ballot et al., 1989a, 1989b). The choice of food vehicle in each case reflected the dietary habits of the population. As anticipated it was not possible to meet all of the criteria for an ideal food vehicle in each trial. Sugar may not have been an optimal choice in Guatemala (Viteri et al., 1983): Although used by a high proportion of the population, considerable variation in both individual and regional consumption was reported; the masking qualities were not perfect and slight yellowing of the sugar was apparent. Masala was used in the South African trial to target iron fortification because this is a condiment used largely by the iron-deficient South African Indian community and not by other segments of the population, some of whom are iron overloaded (Ballot et al., 1989b).

Although each trial included a control group that did not receive the fortified vehicle, only the South African masala trial was double-blinded. The design of the Central American trial (Viteri et al., 1983) presented several problems. First, the four communities chosen were not strictly matched for iron status, which made the choice of a control community difficult. Second, the distribution of the fortified sugar depended on the purchase of sugar by the subjects themselves. Compliance with the fortification program was poor in two communities, probably because some sugar mill workers received free unfortified sugar. These factors may have had some bearing on the disappointing results of this trial.

NaFeEDTA-fortified fish sauce (Garby and Areekul, 1974). Fortified fish sauce was provided to the population of a Thai village for 1 year. Packed red cell volume (PCV) values measured before and after the fortification program showed a significant increase as compared with a control village supplied with unfortified fish sauce (Table 12). The largest mean change (+4.67) was seen in a subgroup of women who were

Table 11 Outline of Field Trials Using NaFeEDTA to Fortify Various Food Vehicles

	Garby and Areekul (1974)	Viteri et al. (1983)	Ballot et al. (1986b)
Geographical region	Thailand	Central America	South Africa
Population studied	Two rural villages	Four rural Guatemalan communities	Urban Indian community in a municipal housing estate
Design of trial	Controlled (one village); not blinded	Controlled (community #15), not blinded	Controlled (random allocation by families); double-blinded
Sample studies	Test village (284) Control village (330)	#13 (186), #14 (306), #15 (234), #16 (296); severely anemic subjects treated before trial	263 families (672 subjects), 129 control families, 134 fortified familes; Hb < 9 g/dL excluded
Food vehicle	Fish-sauce (salt substitute) 30 g NaCl/L, 10 mg Fe/L distributed by village head-man as required	Off-white sugar distribution: sold to store keepers; purchased by participants (poor compliance #13 and #14)	Masala (curry powder) distributed directly to families monthly; free of charge
Consumption of food vehicle (per day)	10–15 mL/person	33 g/person; children highest consumption	5.5 g/person
Fe absorption (%)	8	8	10
Level of fortification and intake	1 mg Fe/mL; 10–15 mg Fe/person per day	13 mg Fe/100 g; 4.29 mg Fe/person per day	1.4 mg Fe/g; 7.7 mg Fe/person per day
Acceptability	No changes	Barely perceptible yellowing	Slight darkening of food
Duration of trial	12 months	20 months	24 months
Abnormal iron status before trial (%)	30–50 of population anemic; 34 initial PCV below normal	Comm / Low PCV / Low sat / Low ferr #13 / 31 / 34 / 52 #14 / 43 / 58 / 72 #15 / 35 / 12 / 37 #16 / 21 / 23 / 34	Females / Males IDA / 24 / 4 ID / 53 / 24
Measurements taken	Packed cell volume (PCV)	Hemoglobin, PCV, %Sat, FEP, Serum Ferritin, Cu, Zn	Hemoglobin, %Sat, Serum Ferritin

IDA = iron deficiency anemia, ID = iron deficiency, % Sat= % saturation of transferrin, FEP = free erythrocyte protoporphyrin, PCV = packed cell volume, Comm = Community, ferr = Ferritin.

Table 12 Initial Values and Mean Changes in PCV in Control and Test Villages After 1 Year of Fortification

	Control village				Test village			
	Number	Initial value	Mean change	SE	Number	Initial value	Mean change	SE
Children	126	36.9	-0.02	0.18	117	36.7	+1.38	0.19
Men	66	40.1	-0.02	0.40	98	40.1	+1.45	0.33
Women	92	36.1	+0.22	0.32	115	36.1	+1.56	0.31

Source: Garby and Areekul (1974).

anemic at the start of the trial (initial PCV < 33). Although a similar subgroup of women in the control group also improved during the year (mean change +2.13) the increase in PCV in the fortified group was significantly greater. The same pattern was seen in both men and children with low initial PCV values.

The increase of 4.67 PCV units over initial values represents an increase of about 187 mg in total body iron, or an increase in daily absorption of about 0.5 mg over the trial. This is 64% of the expected increase in body iron of 0.8 mg/day calculated on the basis of an anticipated absorption of 8% and an assumed daily intake of 10 mL fortified fish sauce (10 mg iron). Iron stores were not measured in the trial and the calculation does not take into account iron laid down in stores. The calculated value therefore is an underestimate of the iron actually absorbed. It nevertheless illustrates that fortification with NaFeEDTA is a highly effective method for improving iron status. Overall, this trial demonstrated that fortification of fish sauce at modest concentrations using NaFeEDTA is feasible and that it can produce a significant improvement in iron status as assessed by a simple criterion (PCV).

NaFeEDTA-fortified sugar (Viteri et al., 1983). The design of this trial makes interpretation of the results difficult. The analysis is based on the comparative changes in iron status observed in four communities. Three (#13, #14, and #16) were test sites and one (#15) was a control site. Measurements of the initial iron status in individuals drawn from test community #14 were significantly worse than the other test communities and the control community (#15). Unfortunately, compliance was poor in this community and also in test community #13. Furthermore, 70% of the families in test communities #13 and #14 used fortified sugar only half the time, and the remaining 30% used it 80% of the time. Finally, subjects with severe anemia were given therapeutic iron to improve their iron status before the trial. Despite these confounding factors, hemoglobin values rose in both males and females after 20 months of fortification, although statistical significance was not attained. Only the children

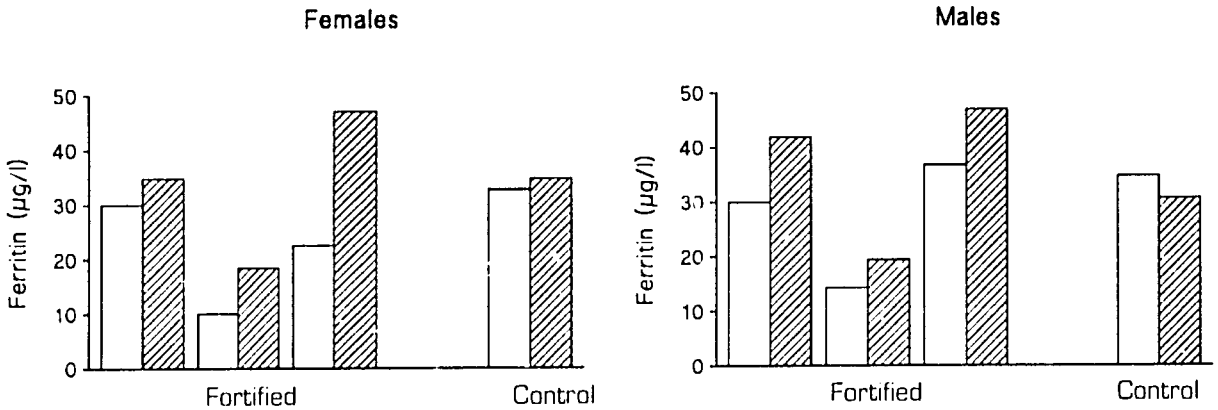

Figure 5

Changes in serum ferritin before (open bars) and after 20 months (cross-hatched bars) of iron fortification. Results are the means from samples drawn from four communities. There was a significant rise in serum ferritin in the three fortified communities (first three groups), but not in the control community. (Source: Viteri et al., 1983; with permission of Academic Press.)

(5-12 years) in communities #13 and #16 showed a significant improvement in hemoglobin concentrations when compared with children in the control community #15 (+2.2 ± 1.7 and +2.2 ± 1.5 g/dL, respectively, vs. + 1.6 ± 1.2 g/dL). The greater benefit observed in children may have resulted because sugar consumption related to body weight was greater in children than in adults. The mean serum ferritin concentration, which is a measure of the size of iron stores, increased in each of the test communities but not in the control community (Figure 5).

The relatively modest improvement in iron status noted in this trial may also have been due, in part, to the fortification amount being considerably less than that in the other two trials (4.3 vs. 10-15 and 7.7 mg iron/person per day).

NaFeEDTA-fortified masala (Ballot et al., 1989b). The design of the most recent fortification trial differed from the earlier studies in that it was conducted in a single community with families randomly assigned to control and test groups. The groups were matched for iron status. It was also double-blinded and care was taken to ensure that crossover between groups did not occur. Fortified or unfortified masala was distributed directly to each family. In addition to evaluating the usual monitors of improving iron status (increasing hematocrit or hemoglobin and ferritin), an attempt was made to estimate the total body iron in each individual by using a composite of the hemoglobin concentration, percent transferrin saturation, and the serum ferritin concentration (Cook et al., 1986). This comprehensive index of iron nutrition made it possibly to compare subjects with wide variations in iron status and thus to assess both the beneficial and potentially adverse effects of additional iron, i.e., development of iron overload (Ballot et al., 1989b).

A significant improvement in body iron as assessed by the index was detectable in the group of women receiving fortified masala after 1 year of the program (Figure 6). This improvement continued during the second year, when the rise in hemoglobin concentration became significantly greater than that in the control group. The

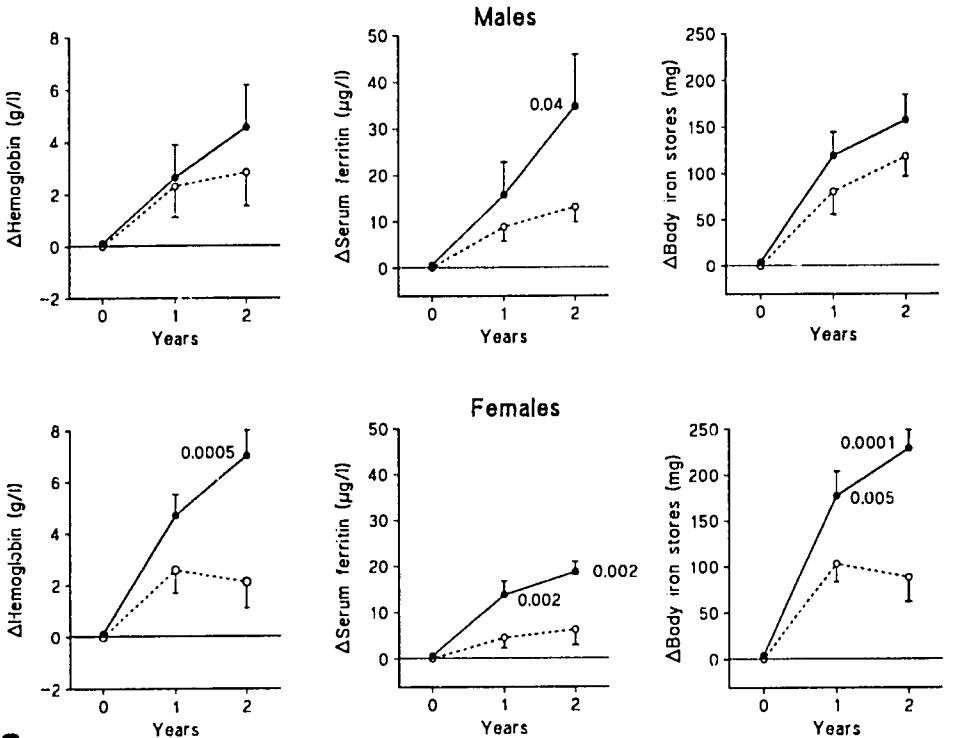

Figure 6

Changes (Δ) in measurements of iron status after 1 and 2 years of iron fortification in fortified (•) and control (o) groups of males and females (mean ± SE). The probability (one tailed, Student's test) that individual changes were greater in the fortified group than in the control group are also shown. (Source: Ballot et al., 1989; with permission.)

Table 13 Prevalence (%) of Iron Deficiency at the Start (Year 0) and After 2 Years of Fortification

Group	No.	Year	Normal	DIS	IDE	IDA	Chi^2	*P*
Females								
Control	161	0	49.1	16.2	17.4	17.4		
		2	62.9	11.2	12.6	13.3	5.9	>0.1
Fortified	164	0	48.8	20.1	9.1	22.0		
		2	78.4	6.8	9.9	4.9	39.5	<0.001
Males								
Control	139	0	74.1	14.4	11.5[a]			
		2	85.4	7.3	7.3[a]		5.6	>0.05
Fortified	134	0	74.6	11.9	13.4[a]			
		2	93.1	3.1	3.8[a]		16.7	<0.001

[a]Categories combined because of small numbers.
DIS: deficient iron stores, IDE: iron deficient erythropoiesis, and IDA: iron deficiency anemia.
Source: Ballot et al. (1989b).

prevalence of iron deficiency dropped dramatically in the women receiving fortified masala (Table 13). Iron deficiency anemia was detected in 22% of individuals at the start of the study but only in 4.9% after 2 years of fortification. The most significant improvement in iron status was noted in women who entered the trial with iron deficiency and especially in those with anemia. Those with anemia showed an increase in calculated body iron of 505 mg, which is equivalent to the absorption of an additional 0.7 mg iron/day. The latter figure is close to the predicted improvement in iron balance of 0.8 mg/day based on isotope absorption studies using NaFeEDTA-fortified masala (Lamparelli et al., 1987).

In iron-replete males the rise in calculated body iron was modest and reached statistical significance only in alcohol abusers receiving fortified masala. This suggests that iron-replete males are unlikely to accumulate excessive amounts of iron under these fortification conditions.

Conclusions

NaFeEDTA appears to be particularly well suited to the conditions encountered in the three trials that have been described, because the traditional diets consumed in these communities tended to be cereal and legume based and were of low intrinsic iron bioavailability because of the presence of phytates and polyphenols (Bothwell and MacPhail, 1992).

The vehicles used in the three studies, sugar in one and condiments in the other two, were particularly appropriate to the eating habits of the groups studied. However, wheat flour and other cereals are equally suitable potential vehicles for NaFeEDTA and the feasability of using wheat flour has already been established in a pilot study with Egyptian bread (el Guindi et al., 1988).

Although NaFeEDTA is not devoid of organoleptic problems, it performs better than many other iron compounds. It caused slight discoloration of the sugar and curry powder used in the field trials that we described but did not affect the consumer acceptability of the products. However, it is not a suitable fortificant for all foods. For example, it has been found that banana-containing weaning cereals and chocolate drinks develop a bluish grey discoloration when NaFeEDTA is added (R.F. Hurrell, unpublished data, 1992). On the other hand, wheat and other cereals appear to be ideal vehicles for NaFeEDTA. Unlike ferrous sulfate, which catalyzes fat oxidation and thus produces unpleasant odors and flavors, NaFeEDTA causes no changes. Wheat flour fortified with NaFeEDTA has been stored for long periods of time without causing organoleptic problems (R.F. Hurrell, unpublished data, 1992).

V. General Conclusions

1. The potential advantages of NaFeEDTA as a food fortificant together with its demonstrated efficaciousness in a recent well-designed field trial prompted a reappraisal of available information relating to its chemical, physiological, and toxicological properties.

2. When NaEDTA is added to food, some of the intrinsic food iron forms complexes with the EDTA to form NaFeEDTA in the stomach. Similarly, when NaFeEDTA is added to food, there is exchange between the iron moiety and other iron in the meal.

3. Iron bound to EDTA is released to physiological mechanisms at the intestinal cell surface, with the amounts absorbed being controlled by the body's requirements. The EDTA then forms complexes with other metals in the intestinal lumen. Only a very small fraction (<1%) of the iron administered as NaFeEDTA is absorbed as the intact NaFeEDTA complex.

4. Up to 5% of the EDTA moiety in any meal is absorbed. This occurs regardless of the EDTA-metal complex administered and is an unregulated process. With NaFeEDTA, most of the EDTA is absorbed after exchange of the iron with other metals, such as zinc and copper. After absorption the EDTA-metal complex is completely eliminated unchanged in the urine within 24–48 hours.

5. Human absorption studies have shown that the iron in NaFeEDTA added to inhibitory meals is two to three times as well absorbed as iron added as ferrous sulphate. However, the absorption of iron from NaFeEDTA is no better than that from ferrous sulphate when added to meals of high bioavailability that contain significant quantities of ascorbic acid or meat.

6. NaFeEDTA in the dose range proposed for food fortificants has no toxic side effects. When NaEDTA is present in food, NaFeEDTA is formed in the stomach. The widespread use of Na_2EDTA and $CaNa_2EDTA$ as additives by the food industry is not associated with any untoward effects.

7. As indicated above, iron and EDTA absorption from NaFeEDTA are independent processes. Sufficient information about the safety of both substances at proposed fortificant concentrations is already available to recommend the use of NaFeEDTA as a food fortificant. Further toxicological studies are not necessary.

8. Concern has been expressed about the use of NaFeEDTA for prolonged periods of time, because exchange of the metal moiety could theoretically lead to trace element depletion. Exchange of iron for zinc is probably the predominant reaction in the lumen of the small intestine. The effect of EDTA on zinc balance depends on the EDTA:zinc molar ratio in the diet. The use of NaFeEDTA in the fortification range proposed (10 mg iron, 67 mg EDTA/person per day) has no detrimental effect on zinc balance. Zinc absorption and zinc balance may actually be improved if the diet has a low zinc content or low zinc bioavailability.

9. There is currently no commercial source of food-grade NaFeEDTA. However, NaFeEDTA is extensively used in agriculture and the processes used for its manufacture are well standardized.

10. NaFeEDTA is an attractive option for delivering fortification iron because

- it is a light colored compound,
- it can be added to several potential food vehicles without affecting the organoleptic properties of the vehicle,
- it is a form of bioavailable fortification iron,
- it promotes the absorption of the intrinsic food iron from low-bioavailability meals, and
- its enhancing effect on iron absorption is not affected by storage conditions or food preparation.

11. Three field trials have demonstrated that fortifying the diet with NaFeEDTA has a significant beneficial effect on iron status.

12. The estimated cost of NaFeEDTA fortification is approximate because of the current absence of a commercial source of food-grade material. Estimated cost is 6 times that for ferrous sulphate for adding equivalent quantities of iron. However, when the improved bioavailability (a factor of 2-3) is taken into account, the cost is only 1.5-3 times as much as ferrous sulphate.

13. The implementation of iron fortification with NaFeEDTA would not require extensive further research. Sufficient information about its potential benefit in various dietary situations is already available to allow for the selection of suitable food vehicles.

14. It might be satisfactory under some circumstances to fortify foods with a soluble iron salt and Na_2EDTA or possibly $CaNa_2EDTA$, because NaFeEDTA would be formed in the stomach. This would require that the iron salt be added to the food item without rendering it organoleptically unacceptable. It might also be necessary to add both the EDTA chelate and the iron salt before processing to ensure adequate mixing. This could be an advantage, since preliminary evidence suggests that a EDTA:Fe molar ratio of 0.5:1 in diets results in maximal iron absorption in diets of low-iron bioavailability. Furthermore, the use of a cheap, soluble iron salt together with a smaller quantity of the more expensive chelator may reduce the cost.

15. Na_2EDTA and $CaNa_2EDTA$ are used extensively in the food industry. JECFA has recently reached the conclusion that NaFeEDTA is also safe when used in supervised food fortification programs in iron-deficient populations and given provisional approval for its use.

VI. Recommendations

1. Sufficient information derived from toxicological studies, human absorption tests, and field fortification trials is available to recommend the use of NaFeEDTA as a food fortificant to improve iron status. Supporting this contention is the recent JECFA provisional approval of NaFeEDTA as safe in food fortification programs in populations in which iron deficiency is endemic.

2. Fortification with NaFeEDTA is expected to be most efficacious in iron-deficient populations subsisting on cereal and legume-based diets.

3. The food vehicles most suited to fortification with NaFeEDTA include wheat flour and other cereal products, soybean and other legume products, and condiments.

4. The following factors merit consideration when establishing the levels at which food should be fortified:

- a reduction in the usually recommended fortification (10 mg iron/person per day) may be possible because iron in the form of NaFeEDTA is two to three times more bioavailable from an inhibitory diet than is iron added as a simple soluble iron salt, such as ferrous sulfate or ferrous fumarate, and absorption of the intrinsic food iron in the meal is also enhanced.
- The dose of EDTA may be the limiting factor in fortifying foods eaten by younger children because NaFeEDTA contains only 15% iron by mass and the ADI for EDTA derived from all sources is 2.5 mg/kg.

5. The possibility of using a soluble iron salt together with Na_2EDTA or $CaNa_2EDTA$ in a EDTA:Fe molar ratio of 1:1 or less should be explored as an alternative to NaFeEDTA for the fortification of foods that do not require prolonged storage and would thus not be rendered organoleptically unsuitable by presence of the iron salt. This approach may have the added advantage of reducing the quantity of EDTA consumed, for example, in foods eaten by young children.

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