EVALUATION OF THE HEALTH ASPECTS OF IRON
AND IRON SALTS AS FOOD INGREDIENTS

JUNE, 1973
Withdrawn
of Superseded

Prepared for
Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D. C.

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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) food substances that are being made by the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) under contract with the Food and Drug Administration (FDA) of the U. S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office, established in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to make a continuing review, analysis, and evaluation of the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. Members of the Select Committee on GRAS Substances who have contributed to this report are named in Section VII. The Select Committee's evaluations are being made independently of FDA or any other governmental or nongovernmental group.

These reports are approved by the Select Committee prior to submission to FDA. Although most LSRO consultants are members of FASEB constituent societies, the reports do not necessarily reflect the views of the Federation as a corporate body or carry the endorsement of the members of its constituent societies.

C. Jelleff Carr, Ph.D., Director
Life Sciences Research Office
FASEB
I. INTRODUCTION

Under terms of FDA Contract 72-85, FASEB's Life Sciences Research Office was requested to evaluate the health aspects of using iron and iron salts as food ingredients, primarily on the basis of information contained in a monograph furnished by FDA (1), summarizing the world's scientific literature from 1920 through 1970, and in certain supplemental documents, including current literature citations obtained through Toxline* and Medline*, available as of June, 1973. Iron and iron salts are food substances that have been generally recognized as safe (GRAS) under the provisions of the Code of Federal Regulations (21 CFR 121.101, revised April 1, 1973).

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the requirement of premarketing clearance for food additives. It is stated in 21 CFR 121.1 that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing, or for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. It is recognized further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety the Select Committee in accord with FDA's guidelines, is relying primarily on the absence of substantive evidence of or reasonable grounds to suspect a significant risk to the public health, and realizes that a conclusion based on such reasoned judgment, is expected even in instances where the available information is qualitatively or quantitatively limited. The Committee is also aware that biological testing, like all of science, is dynamic. Accordingly, the Committee's conclusions, based as they are on the information now available, cannot anticipate and be guided by experiments not yet done or by the results of tests that may be recondected, using new technologies that are continually being evolved. These

*Nationwide online bibliographic retrieval systems initiated by the National Library of Medicine, Bethesda, Maryland.
conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on iron and certain iron salts and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Iron is a natural constituent of foods of plant and animal origin. It occurs as iron, inorganic and organic salts, and as complexes such as heme iron. Food iron content is readily determined, after ashing, by standard procedures (2). Iron in water and soil is usually present as ferric hydroxide, oxides, and oxide complexes.

Because of variations in iron content of foods and the uncertainties of physiological availability, it is common practice to fortify certain types of foods with iron salts to insure that they provide the iron that is necessary to meet nutritional requirements for growth and maintenance.

The Code of Federal Regulations (3) lists the following iron salts and forms of elemental iron as GRAS: ferric phosphate, ferric pyrophosphate, ferric sodium pyrophosphate, ferrous gluconate, ferrous lactate, ferrous sulfate, iron ammonium citrate, iron carbonate, iron chloride, iron oxide, iron (reduced), and iron sulfate.

Some relevant properties of the several GRAS iron preparations used in food, taken mainly from the Food Chemicals Codex (4), are:

Ferric phosphate, FePO₄·xH₂O is specified in the Codex to be not less than 26 percent and not more than 30 percent Fe, with limits on arsenic and other heavy metal impurities. The water of hydration varies from two to four molecules. Ferric phosphate salts are insoluble in water but soluble in mineral acids.

Ferric pyrophosphate, Fe₄(P₂O₇)₃·xH₂O is specified as not less than 24 percent and not more than 26 percent Fe with limits with respect to impurities such as arsenic and heavy metals. The common form is the nonahydrate. It is insoluble in water but soluble in mineral acids.

Ferrous gluconate, C₁₂H₂₂FeO₁₄·2H₂O, is specified by the Codex to be 95 percent of C₁₂H₂₂FeO₁₄ with no more than 2 percent ferric iron.
Limitations on arsenic and heavy metals are stated. It is soluble in water.

Ferrous lactate, \( \text{Fe(C}_5\text{H}_5\text{O}_3)_2 \cdot 3\text{H}_2\text{O} \), is not listed in the Codex but is available as a chemically pure salt for food supplementation. The trihydrate salt is soluble in water.

Ferrous sulfate, \( \text{FeSO}_4 \), occurs anhydrous, as a dihydrate, and as a heptahydrate. The Codex specification lists the heptahydrate as not less than 99.5 percent and not more than the equivalent of 104.5 percent of the heptahydrate, with limits of impurities. Ferrous sulfate salts are soluble in water.

Iron, electrolytic, is elemental iron obtained by electro-deposition in the form of an amorphous grayish-black powder. The Codex establishes limits for purity: not less than 97.0 percent Fe, and not more than 4 ppm arsenic, 20 ppm lead, and 2 ppm mercury.

Iron, reduced, is metallic iron prepared in the form of crystalline particles fine enough to pass through a 100-mesh sieve. It is specified in the Codex to be not less than 96 percent of Fe.

Sodium ferric pyrophosphate, \( \text{Na}_3\text{Fe}_4(\text{P}_2\text{O}_7)_5 \cdot x\text{H}_2\text{O} \), is specified as not less than 14.5 percent and not more than 16 percent Fe, with limits with respect to impurities. This is a complex salt of sodium and ferric pyrophosphates, insoluble in water but soluble in HCl.

Elemental iron for food use is prepared by reduction of heated ferric oxide by hydrogen or carbon monoxide, by decomposition of iron pentacarbonyl, or by electrolytic deposition. Metallic iron, as well as ferrous salts, oxidizes slowly in moist air.

Iron and iron salts have been used as medicines for centuries. Since 1941 iron salts have been added to flour and cereal products to meet adult nutritional requirements (5). It is recognized that the diet of normal adult males contains adequate amounts of iron for their nutritional needs; however, the diet does not contain an adequate amount of iron to meet the nutritional needs of menstruating females. The standards for enrichment state that the supplemental iron shall be a source that is "harmless and assimilable"(5). No improvement in food texture or palatability is reported from the addition of iron salts.

According to a National Research Council Subcommittee survey (6), iron and iron salts are used to fortify products in the indicated percentages:

Ferric phosphate is present in the following foods, arranged approximately in decreasing order of iron content from 0.13 to 0.001 percent:
breakfast cereals, imitation dairy products, nonalcoholic beverages, gravies, grain products such as pastas and rice dishes, baked goods, milk products, and condiments and relishes.

**Ferric pyrophosphate** is present in the following foods arranged approximately in decreasing order of iron content from 0.2 to 0.003 percent: breakfast cereals, sweet sauces, reconstituted vegetable protein, and milk products.

**Ferrous gluconate** is used principally in condiments and relishes to the extent of 0.002 percent.

**Ferrous sulfate** is added to foods in the range of 0.275 to 0.001 percent in the following categories arranged approximately in decreasing order of iron content: nonalcoholic beverages, infant formulas, breakfast cereals, instant tea and coffee, milk products, grain products such as pastas and rice dishes, gelatin puddings, meat products, and baked goods.

**Reduced iron** is added to the following food categories in the range 0.1 to 0.002 percent arranged approximately in decreasing order of iron content: infant cereals, breakfast cereals, infant formulas, grain products such as pastas and rice dishes, and baked goods.

**Sodium ferric pyrophosphate** is present in the following foods arranged in a decreasing order of iron content from 0.5 to 0.002 percent: breakfast cereals, sweet sauces, milk products, baked goods, grain products such as pastas and rice dishes, poultry, fish products, infant formulas, and meat products.

FDA has proposed to increase the level of iron in enriched baked wheat flour products (7).

The Select Committee has no data available on the levels of ferrous lactate used in foods, on iron ammonium citrate, iron carbonate, iron chloride, or iron oxide employed as trace minerals added to animal feeds, or on ferric sulfate which may migrate from paper and paperboard products used in food packaging.

### III. CONSUMER EXPOSURE DATA

The National Research Council survey (6) has supplied information on the possible daily human intakes of iron and certain iron salts added to the total diet for individuals in various age groups (Table I).
### TABLE I

POSSIBLE DAILY INTAKE OF IRON SALTS EXPRESSED AS IRON

<table>
<thead>
<tr>
<th>Age group</th>
<th>Average, mg</th>
<th>Maximum, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ferric phosphate</td>
<td>Ferric pyrophosphate</td>
</tr>
<tr>
<td>0-5 mos.</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>6-11 mos.</td>
<td>9.1</td>
<td>4.0</td>
</tr>
<tr>
<td>12-23 mos.</td>
<td>12.7</td>
<td>4.8</td>
</tr>
<tr>
<td>2-65+ yrs.</td>
<td>16.5</td>
<td>4.6</td>
</tr>
</tbody>
</table>
These figures should be considered in respect to the total quantity of iron used in foods. The NRC subcommittee has pointed out that its calculations of intakes in most cases are overstated, often by considerable margins. * That this is probably true in the case of iron is supported by the following calculation: The NRC subcommittee has provided data indicating that the use of iron in all forms for food purposes in the United States was 367,414 kg (as Fe) in 1970 (6). This figure is reported to comprise between 60 and 70 percent of the total poundage used in food. On the basis of 60 percent adjusted to 100 percent (612,357 kg), and a U.S. population of 200 million, the per capita per day average intake would be 8.4 mg of Fe rather than the adult average daily intake of 155.7 mg given in the table.

These amounts of additive iron are significant when considered in relation to the natural iron content of the diet as can be seen by comparison with calculations on the contribution of each of twelve food categories to the total daily intake of iron as provided by a U.S. Department of Agriculture publication (8). Table II, compiled from these data, shows the maximum average daily intake of iron of males, age 20-34, to be 17.9 mg per day. Other estimates give the apparent average daily intake of iron by adults calculated on a similar distribution of foodstuffs, as 17 mg per day for males and 9-12 mg per day for females (9). The proposed FDA regulation (7) would increase the iron content of enriched flour from 12.0-16.5 mg to 40 mg per pound and that of enriched bread, buns, and rolls from 8.0-12.5 mg to 25 mg per pound. By using the quantities of such products consumed daily as reported by the U.S. Department of Agriculture (8) the iron intake for the same sex-age group would be 21.5 mg per day for males and 13.4 mg per day for females. In contrast, the NRC data (6) list an average of 155.7 mg and a maximum of 397.4 mg of iron as being furnished per day to the 2-65+ years age group.

On the basis of the above considerations, therefore, the Select Committee believes that the NRC subcommittee consumption levels are

*An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (6). The Select Committee finds this explanation reasonable, and concurs in the first recommendation in Section XII of the same report, that "In order to conduct a more accurate survey on the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."
TABLE II

CONTRIBUTION OF DIFFERENT FOODS IN THE U. S. DIET TO IRON INTAKE OF NORMAL ADULTS*

<table>
<thead>
<tr>
<th>Food categories</th>
<th>Contribution to iron intake, percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td>(20 - 34 yrs)</td>
</tr>
<tr>
<td>Meat, poultry, fish</td>
<td>46.3</td>
</tr>
<tr>
<td>Grain products</td>
<td>23.3</td>
</tr>
<tr>
<td>Eggs</td>
<td>6.7</td>
</tr>
<tr>
<td>Other vegetables, fruit</td>
<td>6.6</td>
</tr>
<tr>
<td>Legumes, nuts</td>
<td>4.5</td>
</tr>
<tr>
<td>Potatoes</td>
<td>3.7</td>
</tr>
<tr>
<td>Beverages other than milk and fruit juice</td>
<td>2.5</td>
</tr>
<tr>
<td>Sugars, sweets</td>
<td>2.1</td>
</tr>
<tr>
<td>Tomatoes, citrus fruit</td>
<td>1.5</td>
</tr>
<tr>
<td>Dark green, deep yellow vegetables</td>
<td>1.0</td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>1.0</td>
</tr>
<tr>
<td>Fats, oils</td>
<td>0.7</td>
</tr>
<tr>
<td>Total daily iron intake (mg)</td>
<td>17.9</td>
</tr>
</tbody>
</table>

*Taken from figures in Tables 1a and 16 (8).
not likely to be achieved by any of the age groups.

Poundage data (Table III) show about a twofold increase between 1960 and 1970 in the total use of iron and iron salts as food supplements.

TABLE III

ANNUAL FOOD USE OF IRON AND IRON SALTS, USA (6)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Annual poundage, comparative basis</th>
<th>Total used, 1970</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1960* lb.</td>
<td>1970* lb.</td>
</tr>
<tr>
<td>Ferrous gluconate</td>
<td>4,000</td>
<td>2,200</td>
</tr>
<tr>
<td>Ferric phosphate</td>
<td>194,200</td>
<td>551,465</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>850</td>
<td>1,506</td>
</tr>
<tr>
<td>Iron, reduced</td>
<td>189,800</td>
<td>330,705</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>154,150</td>
<td>414,860</td>
</tr>
<tr>
<td>Sodium ferric pyrophosphate</td>
<td>404,933</td>
<td>401,983</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*These figures are reported to comprise about 60 percent of the actual usage (6).

There are variations in the amount of iron that different segments of the population absorb from food (10). In addition, the unknown amount ingested in the form of iron "tonics" and vitamin supplements containing large amounts of iron, make the intake from food sources alone, insufficient to assess the possible hazards of iron levels in the U. S. diet.

It is also recognized that the figures calculated for the daily intake of iron are of questionable validity because of the special physiological control mechanisms of iron absorption and metabolism (11). In estimating the possible physiological hazard of iron, it is customary to consider principally the amounts added in food processing.
Moreover, public health surveys reveal the prevalence of so-called iron deficiency states in large segments of the U. S. population (12, 13, 14), especially in women under 55 and infants under 3 years of age.

Finally, it must be recognized that iron absorption is remarkably different in individuals according to their nutritional status. In addition, there are a few individuals, who for undefined reasons absorb iron beyond their needs and add iron to their tissue stores (15).

IV. BIOLOGICAL STUDIES

Absorption and metabolism

The absorption of iron is regulated by the form of iron presented for absorption and the physiological mechanisms which control the absorption process (16). Normal individuals are not subject to a physiological overload of iron occurring naturally in foods, but massive doses of iron salts may exert a toxic effect.

Early conclusions on iron absorption have been considerably revised in the light of studies with radioactive iron (17). The Food and Nutrition Board of the National Research Council, in compiling its Recommended Dietary Allowances, has assumed a 10 percent absorption of the total iron present in the average U. S. diet (9).

It is generally agreed that the iron in foods of animal origin is more readily available to man than the iron in foods of plant origin (18). Because most of the iron in animal tissues is heme iron, food iron may be considered to exist in a non-heme pool and a heme pool, with the latter being more available. Iron in heme compounds is protected from chelating agents which exist in plant food. The chelates often bind the iron so firmly that absorption is inhibited. For example, iron in wheat products is absorbed by normal children only to the extent of about 5 percent (18) and the iron from milk, chicken, and iron-supplemented meals only to the extent of 10-20 percent (18, 19). Harrill (20) observed that non-iron deficient young women absorbed only 4 percent of the iron from bread enriched with ferrous sulfate, and 3 percent from bread enriched with ferric phosphate. Three recent publications (21, 22, 23) describe promising procedures for assessing the iron absorption from mixed foodstuffs.

The absorption of iron from mixtures of plant and animal foods is influenced not only by the presence of chelating substances such as phytates in foods of plant origin but also by the potentiating effects of
ascorbic and several amino acids (17, 24, 25, 26). According to Forth and Rummel (26), there is evidence that massive doses of ionizable iron are absorbed through the mediation of a non-ferritin protein component of the duodenal mucosal cells. This accounts for rapid absorption which is controlled by the ferritin system (26).

Iron is absorbed from the intestinal lumen by combination with apoferritin in the intestinal mucosa, forming ferritin. Ferritin may contain up to 4000 to 5000 atoms of iron per mole, if saturated. Iron is transported in the plasma by a carrier, transferrin, a glycoprotein of molecular weight 86,000 to 93,000, with two iron-binding sites per molecule. Under normal conditions the transferrin is about one-third saturated. Biosynthesis of both carriers, apoferritin and apotransferrin, is stimulated by high iron levels in the diet (27). Recent evidence indicates that once absorbed, there is no difference in iron from various dietary sources.

Plasma transferrin transports iron to storage sites in the liver and spleen as well as to the erythropoietic bone marrow. Iron is stored as ferritin and as hemosiderin. Hemosiderin is aggregated ferritin molecules together with other cell constituents (27). Hemosiderin is insoluble and occurs as visible granules in histologic preparations. As body stores become depleted the granules disappear. Excessive deposits of hemosiderin occur in the liver, pancreas, and other organs in hemochromatosis, along with low grade inflammatory and degenerative changes, especially in the liver.

Iron is distributed in the adult male approximately in the amount of 3 to 4 g, as follows (15): hemoglobin, 65 percent, myoglobin, 3-5 percent, ferritin and hemosiderin, 25-30 percent, and traces in a variety of heme and non-heme iron compounds, especially enzymes. The female has a total body iron nearer to 2.5 g, due to lower blood volume with a lower hemoglobin content; parturition and menstruation result in lower iron stores.

Iron is largely conserved in the body through recycling of iron from senescent erythrocytes through the hemopoietic system. This amounts to a daily turnover of 17 to 24 mg of iron. In the male the principal mode of iron loss occurs through sloughing of epithelial cells, biliary excretion, and loss of erythrocytes into the gastrointestinal tract. These losses are offset by physiologically-controlled absorption of iron by means of the ferritin-transferrin system (27). It is generally agreed that the necessary absorption of iron in the male is about 1.0 mg per day. In children, the replacement requirement varies over the range 0.2-1.0
mg per day. The female has the additional avenue of iron loss through menstruation. This increases the iron replacement requirement for the average adult menstruating female to 2.8 mg per day (28). Ranges for age-sex groups that differ only slightly from those given, have been suggested by the Committee on Iron Deficiency, Council on Foods and Nutrition, American Medical Association, and tabulated by Forth and Rummel in a recent review (26).

There is a long-standing controversy over the relative effectiveness of various iron salts in absorption from the gut. The amount of iron contained in a normal diet is calculated to be about 6 mg per 1000 kcal (9). With an assumed 10 percent absorption of food iron, this meets the needs of normal males but falls short of meeting the needs of infants, children and adolescent boys, or adult females.

In a study with $^{59}$Fe Hallberg and Brise (17) demonstrated that 3 to 7 times more iron was absorbed from a therapeutic dose of ferrous sulfate (30 mg Fe) than from an equivalent dose of ferric sulfate. In the earlier literature, then-unrecognized homeostatic mechanisms frequently were responsible for reports of equivocal results. In a more recent comparison, Fritz et al. (16) used the repletion of hemoglobin and hematocrit in anemic rats and chicks to compare the biological availability of a number of iron compounds. With ferrous sulfate given a standard value of 100, the relative biological values of GRAS iron compounds in rats were as follows: ferrous gluconate, 97; reduced iron, 16-37; sodium ferric pyrophosphate, 11-21, and ferric phosphate, 12-30. The results with chicks were similar. Their observations were in accord with the generally-held view that ferrous salts are better than ferric as iron supplements (17).

**Acute Toxicity (Animals)**

A number of animal studies have documented the toxic potential of the common iron salts. Hoppe (29) compared the LD$_{50}$'s of ferrous sulfate and ferrous gluconate in mice intravenously and orally. The salts were equally toxic when given intravenously and calculated as ferrous iron (10.2 vs 10.8 mg per kg) but orally the gluconate was less toxic (429 vs 306 mg per kg). Similar ratios were obtained in rats.

Since neither the mouse nor the rat has a vomiting reflex, Hoppe (29) used the cat to examine the action of these substances on this reflex. Twice as much iron as ferrous gluconate could be given without inducing vomiting as could iron in the form of ferrous sulfate. Acute lethality from either compound could not be produced in cats by the oral route, as large doses uniformly produced vomiting.
A careful study of the toxicity of several iron salts was published by Weaver et al. (30). A summary of the findings is given in Table IV. Additional acute toxicity data are tabulated in the monograph (1).

**TABLE IV**

**ACUTE TOXICITY OF IRON COMPOUNDS**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Species</th>
<th>Route</th>
<th>LD$_{50}$, mg iron/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous fumarate</td>
<td>mouse</td>
<td>i.p.</td>
<td>157.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oral</td>
<td>516.1</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>oral</td>
<td>&gt;2329.0</td>
</tr>
<tr>
<td>Ferrous gluconate</td>
<td>mouse</td>
<td>i.v.</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.p.</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oral</td>
<td>457.4</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>oral</td>
<td>865.0</td>
</tr>
<tr>
<td></td>
<td>dog</td>
<td></td>
<td>&gt;46.4</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>mouse</td>
<td>i.v.</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.p.</td>
<td>40.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oral</td>
<td>305.0</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>oral</td>
<td>780.0</td>
</tr>
<tr>
<td></td>
<td>dog</td>
<td>i.v.</td>
<td>23.0</td>
</tr>
</tbody>
</table>

Shanas and Boyd (31) studied the toxicity of reduced iron and found to be a hundredfold less toxic than ferrous sulfate, ferrous chloride, or ferrous gluconate. The LD$_{50}$ (as elemental iron) orally in rats was 100 g per kg. Death was apparently due to bowel obstruction at these high doses rather than to cellular damage. When the dose was between 60 and 100 g per kg, death was usually delayed to later than 60 hours and was primarily due to severe gastroenteritis complicated by dehydration, hemoconcentration, and electrolytic imbalance. At doses below 10 g of reduced iron per kg there was no evidence of gastrointestinal irritation. At this level (10 g per kg orally) there was significant iron absorption within 48 hours.
In dogs, ferrous carbonate proved to be less toxic than either ferrous sulfate or ferrous gluconate (32). Ferrous sulfate was fatal in an oral dose of 0.6 g per kg (as ferrous iron) and 0.3 g per kg produced extensive ulceration and inflammation of the stomach and duodenum. Even doses as low as 0.012 g per kg produced some ulceration. Ferrous gluconate was not lethal at 0.75 g per kg but this dose as well as 0.375 g per kg caused gastrointestinal tract damage. In contrast, ferrous carbonate in the amount of 0.5 g iron per kg produced no toxic symptoms or postmortem signs. The highest dose of 3.0 g per kg gave only slight ulceration of the fundus and pylorus. The highest non-toxic oral dose level for ferrous carbonate in dogs was 1.5 g per kg; ferrous gluconate, 0.047 g per kg; ferrous succinate, 0.094 g per kg; and ferrous sulfate, > 0.012 g per kg.

The metabolic and circulatory effects of toxic amounts of iron salts were studied by Reissman and Coleman (33). Dogs given ferrous sulfate, gluconate, or chloride orally (150-750 mg per kg) developed hyperventilation within an hour secondary to a marked metabolic acidosis with blood levels as low as pH 6.7. The authors suggest that this acidosis was mainly due to the hydrolysing effect of ferric ions and partly due to an increase in circulating lactic and citric acids. Respiratory failure was the usual cause of death.

A lethal dose of ferrous sulfate (225 mg iron per kg injected intraduodenally) resulted in a sharp drop in cardiac output (57 percent) of dogs after one hour (34). Simultaneously, there was a 17 percent decrease in blood pressure, a 9 percent decrease in blood volume, and 100 percent increase in total peripheral resistance.

Enzyme histochemical studies have shown that the activities of hepatic enzymes in rabbits (oxidative and glucose-6-phosphatase) were increased 8 hours after administration of toxic amounts of ferrous sulfate (90 mg per kg intravenously) and were decreased at 12 hours as a result of hepatocellular damage (35).

The usual signs and symptoms in animals receiving toxic amounts of iron salts are initial depression, rapid and shallow respiration, coma, convulsions, respiratory failure, and cardiac arrest. Diarrhea and vomiting are prominent (in those species with the vomiting reflex) and delayed deaths are not infrequent (36). Congestion and hemorrhagic areas may be observed along the gastrointestinal tract by postmortem examination. Marked erosion and mucosal gastrointestinal sloughing occur if death is delayed beyond 1 or 2 days. The liver is markedly congested and petechial hemorrhages are often observed in the lungs.
Acute Toxicity (Man)

Cases of acute iron poisoning of young children and adults have been reported in the literature (37-51). Nearly all of the cases involved ferrous sulfate, probably because it is available as sugar-coated pills. Aldrich (52) lists 42 cases of poisoning occurring over a period of about 10 years in children up to 4 years old. As little as 3.0 g was fatal in one case and recovery followed as much as 15 g in another. In England, fatal iron poisoning has been estimated to be caused by as little as 1 g in children (53).

Deaths from ferrous sulfate heptahydrate have occurred after oral ingestion of as little as 40 mg per kg and as much as 1600 mg per kg (36). One adult consumed 56 g of ferrous sulfate in a suicide attempt, and survived after three months of treatment. The average fatal dose in 23 cases (largely children) was approximately 900 mg per kg.

The effects of large toxic doses of iron salts in man are similar to those in animals. Iron preparations cause severe gastroenteritis with hematemesis, abdominal pain and diarrhea, depression and lassitude leading to shock, with possible temporary recovery and subsequent deterioration of the subject's health. The gastrointestinal effects occur after therapeutic dose levels where side effects of constipation, heartburn, diarrhea, nausea, and epigastric discomfort are common (54).

The cause of death in iron poisoning is still debated. It has been suggested that early deaths (4-6 hours) are due largely to a corrosive action on the gastric mucosa resulting in excessive absorption of iron into the circulation. The large amounts of ferritin formed have a vaso-depressor action resulting in shock and death which may occur as a second crisis at 20 to 50 hours (36, 55, 56). If the patient survives this, the massive amounts of iron (circulating levels 10 to 100-fold above normal) poison enzymes and cause general cellular dysfunction which leads to delayed deaths(35).

The combination of ascorbic acid with iron salts enhances absorption from oral doses, and such preparations have greater toxic potential than the same amount of iron without ascorbic acid (25).

Short-term Toxicity (Animals)

Very few studies have employed doses of iron salts administered over weeks or even a few days. Nissim (57) gave various weekly doses of saccharated iron oxide intravenously to mice and rats for periods up to 12 weeks. Rabbits received daily intravenous injections of 45 mg iron per kg up to 12 days. Some animals received a single injection and were
sacrificed at various time intervals thereafter. Total doses as high as 2.16 g per kg in mice and 1 g per kg in guinea pigs were given. Tissue damage was evident by hemorrhagic emboli in the lungs, patchy parenchymal damage in the liver, and adrenal hemorrhage. No cirrhosis of the liver or pancreatic fibrosis was observed.

D'Arcy and Howard (58) gave daily oral doses of several iron salts to dogs for 14 days before necropsy. Ferrous carbonate in doses of 0.25, 0.5, and 1.0 g iron per kg produced no signs of toxicity or histological evidence of damage to the stomach or intestines. Ferrous gluconate was non-toxic at doses of 0.25 g iron per kg, although 0.50 g per kg produced slight inflammation of the fundus and pylorus. Ferrous sulfate produced toxic effects at dose levels of 0.100, 0.050, and 0.025 g of iron per kg. The highest non-toxic dose of this salt was 0.005 g per kg. All dogs had heavy deposits of iron in the spleen.

Hoppe (29) dosed one group of cats orally 5 days per week for 2 weeks with ferrous sulfate (25 to 400 mg iron per kg), and another group with ferrous gluconate (100 to 1600 mg iron per kg). No signs of toxicity were seen with ferrous gluconate, while one of two cats receiving ferrous sulfate (400 mg per kg) died on the 5th day.

**Chronic Toxicity (Animals)**

Classical long-term toxicity studies have not been conducted, but special-purpose experiments involving long-term administration of iron salts are available. Several studies have been attempted to produce hemochromatosis in animals, but have failed (59). Heavy deposits of iron in many tissues can be induced easily (hemosiderosis) but the associated tissue damage of fibrosis of the liver, spleen, and pancreas, which is characteristic of hemochromatosis in humans, does not occur.

Polson (60) gave rabbits "dialyzed iron" in doses of 5 or 10 ml subcutaneously or intraperitoneally at unspecified intervals up to 4 years. The total amount of iron was not specified. Postmortem examinations demonstrated the marked degree of iron absorption and the absence of liver cirrhosis and pancreatic damage in all animals (60).

Rather (61) attempted to produce fibrosis of the liver by long-term feeding of iron to rats. Animals were fed a diet containing 6 percent ferric citrate for 6 to 18 months. At necropsy, livers were engorged with hemosiderin, chiefly in the parenchymal and Kupffer cells. Large deposits of hemosiderin were also found in the spleen, abdominal lymph nodes, pancreas, kidney, and adrenal glands. However, cirrhosis was not found in any of the iron-treated animals.
The longest study reported (62) extended up to 7 years, with the same negative results regarding production of hemochromatosis. This study might be called more properly the long-term effects of a single dose with divided administration. Four dogs were given iron oxide intravenously in weekly injections spread over 6 to 10 weeks until a total dose of 0.5 or 1.0 g iron per kg was achieved. During the observation period, hepatic function tests were done and repeated biopsies of liver, spleen, pancreas, and other organs were made. No hemochromatosis was found after seven years. It is of significance, however, that all dogs developed blindness. The lesions were pathologically similar to retinitis pigmentosa. Details of this experiment are vague and it is not clear that proper controls were carried through the study so that the results could be attributed to the iron administered.

Short- and Long-term Toxicity (Man)

Murphy (63) has described a subject who took 600 mg of ferrous sulfate daily for 19 years without producing abnormal clinical or biochemical changes related to the iron intake. The normal serum iron value (127 μg per 100 ml) and the low percentage saturation (total iron-binding capacity = 448 μg per 100 ml) made hemochromatosis and siderosis unlikely. Apparently the iron mucosal block - a control or feedback mechanism thought to be present in the intestinal mucosal cells that controls the avidity with which iron is absorbed and transferred to the blood (15) - in this patient was quite efficient.

Perhaps the best example of long-term iron intake is provided by a report on South African Bantu tribesmen whose daily intake of iron is calculated to be between 100 and 200 mg (64). Below the level of about 100 mg per day, normal humans have a mucosal block that prevents excessive absorption of iron. The daily diet of various Bantu groups is enough to exceed the protective mucosal block and cause the large deposits of iron found in the organs of the adult Bantu who comes to autopsy. However, it is now generally accepted that iron overload (hemosiderosis) such as the Bantu natives exhibit, is separate and distinct from hemochromatosis which is thought to be a genetically determined disorder.

The iron content of the diet, as changed by a proposed increase in the enrichment level in flour (7), apparently will not affect the accumulation of iron by normal males (15). On the basis that hemochromatosis is an inborn error of metabolism, the present iron content of the diet as enriched by iron additives, or as proposed to be enriched, will not result in an increased incidence of the disease. Some experts are of the opinion that an increase in dietary iron over a period of time would result in an increased rate of accumulation in individuals with the inborn error of metabolism (15).
V. OPINION

The Select Committee recognizes nutritional requirement differences for iron in adult males and females, in pregnant women, and during periods of rapid growth in infancy and adolescence, and that the present natural iron content of the diet is at best marginal and poorly utilized. Although iron is added as a public health measure, the attainment of minimum daily requirements by the population is influenced by the variable factors of absorption, physiological control, state of health, nutrition, and the bioavailability of different forms of iron. The goal of a food intake of iron ten times the requirement, however, could be exceeded manyfold by present manufacturing practices. In addition, many individuals take iron supplements that could produce an overload on the iron metabolism system. Such ingestion of iron can have a toxic effect by direct absorption of ionizable iron which exerts a corrosive effect on the gastrointestinal mucosa and a general systemic toxicity. This raises a question as to the potential risk of using rapidly absorbable salts as a source of iron supplement, particularly in individuals who have an abnormality in the regulation of iron absorption.

It is not known whether individuals with iron absorption defects are subject to further acceleration of their disease by supplemental iron in the diet. It should be noted also that adult females on continuously administered oral contraceptive agents may be less subject to iron deficiency anemia than those not receiving or on cyclic administration of this medication, and may be subject to iron overload because of their reduced loss of iron by menstruation. It is essential that studies be implemented to determine the long-term effects of addition of iron to foods.

Because iron is added to foods only for nutritional purposes, it is necessary to consider the total problem and take into account the various forms and amounts of dietary iron from all sources, including vitamin-mineral supplements and tonics, and the regional nutritional habits of the population. Regular monitoring of total iron intake is essential to establish the kind and amount of iron supplements appropriate to assure adequate dietary iron and still avoid undue risk to those individuals who may be affected with iron storage disorders.
The Select Committee has weighed the foregoing and concludes that:

There is no evidence in the available information on iron or the iron salts considered in this report that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current and in the manner now practiced, including the levels at which bread, buns and rolls are proposed to be fortified. However, without additional data, it is not possible to determine whether a significant increase in their consumption would constitute a dietary hazard.
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