

FERROUS GLYCINATE (PROCESSED WITH CITRIC ACID)

First draft prepared by

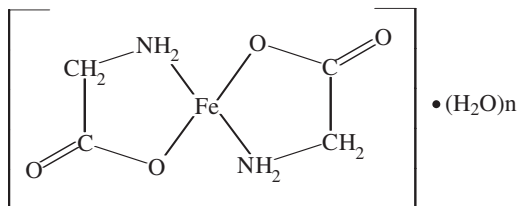
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Explanation	461
Biological data	461
Biochemical aspects	461
Absorption and excretion of iron from ferrous glycinate	464
Bioavailability of iron from ferrous glycinate.....	465
Biotransformation.....	471
Influence of ferrous glycinate on the chemistry of nutrients.....	472
Toxicological studies	472
Acute toxicity.....	472
Short-term toxicity.....	472
Long-term studies of toxicityand carcinogenicity.....	474
Genotoxicity	474
Reproductive toxicity.....	474
Observations in humans.....	474
Ferrous glycinate supplementation	474
Ferrous glycinate-fortified foods.....	478
Comments	482
Evaluation	482
References	483

1. EXPLANATION

The call for data for the sixty-first meeting referred to this substance as ferrous bisglycinate. The Committee decided that this name did not accurately describe the substance being evaluated and therefore agreed that it should be referred to as “ferrous glycinate (processed with citric acid)”. Ferrous glycinate (processed with citric acid) is an iron [II] chelate with the amino acid glycine, and also contains citric acid. It is manufactured by the reaction of reduced iron with glycine, in the presence of citric acid. At chemical equilibrium, > 97% of the ferrous ions are chelated. The resulting product is spray-dried without prior removal of the citric acid. The substance is highly hygroscopic and may contain water in variable amounts.

At its twenty-seventh meeting (Annex 1, reference 62), the Committee allocated a provisional maximum tolerable daily intake of 0.8mg/kg bw for iron from all sources, except for iron oxides used as food colouring agents, supplemental iron taken during pregnancy or lactation, and supplemental iron for specific clinical requirements. At its present meeting, the Committee was asked to comment

Figure 1. Structural formula of ferrous glycinate

on the safety of ferrous glycinate as a source of iron for dietary supplementation and as a fortificant for general use in food products.

The State of São Paulo, Brazil, has mandated the use of milk fortified with Ferrochel[®] ferrous glycinate in State-supported assistance programmes for the prevention and treatment of iron-deficiency and iron-deficiency anaemia in young children (Pineda, 2001). Other countries that currently use Ferrochel[®] ferrous glycinate for the fortification of the iron content of foods include seven in Latin America (Argentina, Chile, Colombia, Ecuador, Mexico, Paraguay, Venezuela), two in Europe (Italy, Spain), and one in Asia (Thailand), as well as Saudi Arabia and South Africa (Pineda, 2001; Allen, 2002).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

Ferrous glycinate consists of one molecule of ferrous iron covalently bound to two molecules of glycine (see Figure 1) (Ashmead, 2001; Allen, 2002). The iron is bound to the carboxyl group and the α -amino group of glycine by coordinate covalent bonds, to form two heterocyclic rings (Atkins & Beran, 1992; McMurry & Fay, 1995). This 1:2 metal to ligand ratio restricts reaction with dietary inhibitors of iron absorption, neutralizes the valence of the ferrous iron, and protects gastrointestinal surfaces from irritation by the iron (Jeppsen, 2001; Allen, 2002). This structure makes ferrous glycinate an ideal fortificant for foods with a high content of absorption inhibitors such as phytate (Jeppsen, 2001).

The commercial food-grade formulation of ferrous glycinate is called Ferrochel[®] ferrous glycinate, and consists of 77% ferrous glycinate chelate, 17% citric acid, 4% moisture, 2% maltodextrin, and < 0.1% silicon dioxide by weight. The chelate itself consists of 27% iron and 73% glycine by weight, while 20% iron is present in the hydrated form of the compound. All components of Ferrochel[®] ferrous glycinate are acceptable food additives that meet appropriate food-grade specifications.

The solubility of iron from Ferrochel[®] ferrous glycinate is not affected by changes in pH (e.g. from pH 2 to pH 6) (García-Casal et al., 1997; García-Casal & Layrisse, 2001). Ferrochel[®] ferrous glycinate has weak pro-oxidant properties and is stable when exposed to air and ambient temperatures (Olivares et al., 1997).

In a study by Hendricks & Ashmead (1995), Ferrochel[®] ferrous glycinate was added to milk and yoghurt at concentrations providing iron at 3mg/l, and to corn flour at a concentration providing approximately 9.1 mg/kg. The milk, yoghurt and corn flour fortified with Ferrochel[®] ferrous glycinate were packaged in sterile containers and stored in a freezer, in a refrigerator, or at room temperature (22°C). Before packaging, Ferrochel[®] ferrous glycinate-fortified milk was also subjected to homogenization and pasteurization. Lipid peroxidation was measured by the presence of thiobarbituric acid-reactive substances (TBARS) in Ferrochel[®] ferrous glycinate-fortified milk and yoghurt after 3, 10, and 18 days of storage, and in the fortified corn flour after 4 weeks of storage. There were no significant differences in bacterial growth or in the quantity of TBARS produced in control and Ferrochel[®] ferrous glycinate-fortified milk and yoghurt after 3, 10 or 18 days of storage, at any of the temperatures examined; however, after 18 days of storage, the control milk had spoiled, while the Ferrochel[®] ferrous glycinate-fortified milk had not. Similarly, no significant differences in lipid peroxidation were noted between control and Ferrochel[®] ferrous glycinate-fortified corn flour after 4 weeks of storage at any of the temperatures examined.

Similar results were reported by Umbelino et al. (2001) after examination of the organoleptic characteristics (fermentation time, pH, titratable acidity, viscosity, consistency, iron concentration and sensory properties) of soya bean yoghurt fortified with iron at 12mg/l as Ferrochel[®] ferrous glycinate, with no alterations in fermentation time, titratable acidity or the sensory and rheologic properties of the soya bean yoghurt.

The organoleptic characteristics of margarine fortified at a concentration of 20 or 60mg/kg with Ferrochel[®] ferrous glycinate were evaluated every 30 days for 120 days (Name, 1996). Control margarine received a rating of "good" upon evaluation on days 30, 60, and 90 of the study, and a rating of "bad" on study day 120. Margarine fortified with Ferrochel[®] ferrous glycinate at 20mg/kg was rated "acceptable" up to day 90, and "bad" on day 120 of the study. Conversely, margarine fortified at a level of 60mg/kg received ratings of "acceptable" on days 30 and 60, and ratings of "bad" on days 90 and 120 of the study.

A panel of 15 judges trained in descriptive analyses who evaluated the sensory quality of maize meals fortified with ferrous glycinate providing iron at 30 or 60 mg/kg meal, reported no difference in colour between unfortified meals and meals fortified with ferrous glycinate. The degree of lipid oxidation in the meals was evaluated by measuring of hexanal production after storage. Maize meals fortified with ferrous glycinate and stored for 20 days at temperatures of 30, 40, or 50°C were more rancid (as reflected by high hexanal production) than unfortified maize meals, or those fortified with iron sulfate (FeSO₄), ferric trisglycinate, or NaFe-EDTA. The addition as antioxidants of butylated hydroxyanisole at 100ppm or butylated hydroxytoluene at 200ppm, singly or in combination with citric acid, decreased hexanal production in maize meals fortified with ferrous glycinate (Bovell-Benjamin et al., 1999).

Despite the results of Bovell-Benjamin et al. (1999), 40 infants aged 6–24 months and their mothers found unfortified maize meals and meals that had been fortified with ferrous glycinate, providing iron at 30mg/kg and stored at 30°C for

20 days (Bovell-Benjamin et al., 1998) equally acceptable. Additionally, the acceptability rating of ferrous glycinate-fortified maize meal was not affected by the addition of butylated hydroxyanisole at 50 ppm.

2.2.1 Absorption and excretion of iron from ferrous glycinate

Iron is a normal constituent of the diet and of the body, and is absorbed from the gastrointestinal tract by one of two pathways, depending on whether the iron is in the organic (haem) or inorganic (non-haem) form. Dietary haem iron, which is derived primarily from haemoglobin and myoglobin in meat, is absorbed into the intestinal cells as the intact porphyrin complex (INACG, 1993; IOM, 2001). Iron is released from this complex by the haem oxygenase enzyme and transferred to the bloodstream for transport, together with other iron taken up by the cells (INACG, 1993). Haem iron is highly bioavailable (20–25% absorption) and is relatively unaffected by dietary factors (INACG, 1993; IOM, 2001). Non-haem iron, which is derived from various food sources (e.g. vegetables, dairy products, meat and dietary iron fortificants), is solubilized and transferred into a common pool of non-haem iron located in the lumen of the upper gastrointestinal tract (Cook et al., 1972; INACG, 1993; IOM, 2001; MacPhail, 2001). In contrast to haem iron, the amount of non-haem iron absorbed from this pool is greatly affected by the presence of ligands in undigested or partially digested foods, which either enhance (e.g. ascorbic acid) or inhibit (e.g. polyphenols, phytate) absorption (Cook et al., 1972; INACG, 1993; IOM, 2001; MacPhail, 2001). Additionally, some insoluble forms of iron may be ingested; they therefore do not enter the common non-haem iron pool and are not absorbed (INACG, 1993).

Ferrous glycinate (processed with citric acid) enters the intestinal intraluminal pool of inorganic, non-haem iron. The absorption of ferrous glycinate in the intestine would be expected to follow the general mechanism of absorption for amino acid–metal chelates proposed by Ashmead et al., (1985), whereby ferrous glycinate is absorbed intact *via* the dipeptide pathway and subsequently hydrolysed into its respective iron and glycine components in the intestinal mucosa. In studies in which $^{59}\text{FeSO}_4$ and ^{55}Fe -glycinate (^{55}Fe -Ferrochel[®] ferrous glycinate) were administered together in a whole-maize or wheat-flour meal (Allen et al., 1998; Bovell-Benjamin et al., 2000; Layrisse et al., 2000), significantly more iron was absorbed from ferrous glycinate (or Ferrochel[®] ferrous glycinate) than from FeSO_4 . There was no exchange of radiolabelled iron (i.e. ^{59}Fe and ^{55}Fe) between ferrous glycinate (or Ferrochel[®] ferrous glycinate) and FeSO_4 in the non-haem iron pool prior to intestinal absorption, indicating that ferrous glycinate (or Ferrochel[®] ferrous glycinate) was absorbed intact into the mucosal cells of the intestine (Allen et al., 1998; Bovell-Benjamin et al., 2000; Layrisse et al., 2000).

After absorption into the mucosal cells, the iron is hydrolysed and then distributed, reversibly bound to transferrin, for use in proteins, including storage proteins (e.g. ferritin and haemosiderin), transport proteins (e.g. transferrin and lactoferrin), haem-containing proteins (e.g. haemoglobin, myoglobin, and cytochromes), and enzymes (e.g. iron-containing and activated non-haem enzymes, iron-sulphur enzymes or flavoproteins) (IOM, 2001; MacPhail, 2001). The average total concentration of body iron is approximately 50 and 40 mg/kg bw

in men and women, respectively (Bothwell et al., 1979). Iron in storage proteins makes up about 20–30% of the iron in the body, and primary iron storage sites have been identified in the cells of the liver, spleen and bone marrow. Approximately 60–70% of total body iron is present in the haemoglobin of circulating erythrocytes, while 10% has been identified in myoglobin, cytochromes, and other iron-containing enzymes (IOM, 2001). The amount of iron in the body is highly conserved, with daily basal losses of 0.60 mg in men, 0.64 mg in non-menstruating women, and 1.20 mg in menstruating women (Green et al., 1968). The majority of iron loss has been reported to be a consequence of faecal excretion. Iron losses also may occur in the urine, gastrointestinal tract and skin, which contribute approximately 0.08, 0.60, and 0.20–0.30 mg of the iron, respectively, that is lost from the body per day (Green et al., 1968; IOM, 2001).

After dissociation from ferrous glycinate, the free amino acid, glycine, is used in normal metabolic processes. Glycine, the smallest of the amino acids, is essential for the biosynthesis of nucleic acids and other amino acids, as well as proteins, such as collagen and elastin. In addition, glycine functions as a major inhibitory neurotransmitter in the spinal cord and brainstem of the central nervous system (Lodish et al., 1995; Silverthorn, 1998).

2.1.2 Bioavailability of iron from ferrous glycinate

Ferrous fumarate and ferrous sulfate lose solubility as pH increases toward neutrality (García-Casal et al., 1997). Aided by its high solubility at physiological pH (García-Casal & Layrisse, 2001), ferrous glycinate (or Ferrochel[®] ferrous glycinate) maintains high iron bioavailability in foods, despite the presence of iron absorption inhibitory factors such as phytic acid, which form insoluble complexes with iron (Bovell-Benjamin et al., 2000; Layrisse et al., 2000). The absorption of iron from ferrous glycinate (or Ferrochel[®] ferrous glycinate) is regulated physiologically by the body's iron status; like other iron compounds (Olivares et al., 1997; Allen et al., 1998; Iost et al., 1998; Bovell-Benjamin et al., 2000; Layrisse et al., 2000; Giorgini et al., 2001; Olivares & Pizarro, 2001). Therefore, fortification of foods with ferrous glycinate (or Ferrochel[®] ferrous glycinate) would not be expected to result in iron overload in otherwise normal populations.

Ferrous glycinate (or Ferrochel[®] ferrous glycinate) has been used in numerous studies of absorption conducted to assess iron absorption and/or iron status in healthy volunteers and in populations with low iron status. In several studies, ferrous ascorbate was administered as a reference dose under standardized conditions in order to minimize the effects of individual variations in absorptive capacity (i.e. absorption of iron from a given test meal/carrier food is expressed as a ratio to the reference dose) (Baynes et al., 1987). In each study, the carrier foods used to administer the iron, the level of use, and the resulting exposures to iron have been identified for comparison.

A comparative study of the absorption of iron from ferrous glycinate, ferric tris-glycinate, and iron sulfate in a whole-maize meal was conducted by Bovell-Benjamin et al. (2000). Ten male healthy volunteers (aged 19–30 years) with normal iron status were provided with a porridge meal (consisting of 87.5 g whole-

maize meal in 625 ml boiling water, 50 g dry non-fat milk, 125 g margarine, and 37 g Jamaican brown sugar) fortified with iron at 11 mg/kg maize as $^{59}\text{FeSO}_4$ on day 1, and as ^{55}Fe -glycinate on day 2. The total iron content of the meal was reported to be about 1.9 mg/serving. Fourteen days later (on day 16 of the study), fasting blood samples were obtained from each of the men for measurement of the amount of ^{59}Fe and ^{55}Fe incorporated into erythrocytes. Pre-study blood samples also were obtained for measurement of baseline serum ferritin, haemoglobin, and erythrocyte radioactivity. Assuming that 85% of absorbed radioactivity was incorporated into erythrocytes, iron absorption was calculated from the estimated blood volume of each subject. The geometric mean percentage of iron absorbed from ferrous glycinate (6%) was significantly greater than from FeSO_4 (1.7%) in a whole-maize porridge. After fasting blood samples had been taken on day 16, the men ate a whole-maize porridge fortified with iron (as FeSO_4) at 0.011 mg/g of maize and containing a mixture of 55.5 kBq $^{59}\text{FeSO}_4$ and 111 kBq ^{55}Fe -glycinate. Blood samples were again drawn from the men 14 days later (on day 31 of the study) for measurement of radioactivity incorporated into erythrocytes. The percentage of absorption from each source of iron was unaffected by the simultaneous presence of the respective sources. As reported after consecutive administration, a significantly greater geometric mean percentage of iron was absorbed from ferrous glycinate (6.8%) than from FeSO_4 (1%). Additionally, when the results for the 31-day study period were combined, the average absorption of iron from ferrous glycinate was reported to be significantly higher (4.7-fold) than from FeSO_4 . Plasma ferritin values were reported to be negatively correlated with the percentage of iron absorbed from both sources of iron, which the authors indicated to be suggestive of down-regulation of iron absorption when iron reserves were sufficient. The men who absorbed the least or the most iron from FeSO_4 were also reported to have absorbed the least or most iron from ferrous glycinate.

In a second study by Bovell-Benjamin et al. (2000), 23 healthy, non-pregnant female volunteers (aged 18–48 years) with a wide range of iron statuses were given a dose of ferrous ascorbate reference solution (30 mg ascorbic acid and 3 mg iron as $^{59}\text{FeSO}_4$) before or after drinking a solution containing 3 mg of iron as ^{55}Fe -glycinate in 10 ml distilled water. Baseline blood radioactivity and serum ferritin, and haemoglobin concentrations were determined for each woman from blood samples obtained before treatment. Two weeks later (on day 16 of the study), fasting blood samples were obtained from each woman for measurement of the amounts of ^{59}Fe and ^{55}Fe incorporated into erythrocytes. Complete data were available for only 21 women as one woman withdrew for personal reasons and the data for another woman were discarded because of technical problems. Geometric mean iron absorption from the ferrous ascorbate reference dose (32.5%) was reported to be significantly higher than that from ferrous glycinate (9.1%). After fasting blood samples had been drawn on days 16 and 17, the women were randomly assigned to drink water containing 55.5 kBq ^{59}Fe -trisglycinate or to eat whole-maize porridge fortified with iron (as 111 kBq ^{59}Fe -trisglycinate at 11 mg)/kg of maize. The total iron content of the meal was reported to be 1.863 mg/serving. Blood samples were drawn from the women 14 days later (on day 31 of the study) for measurement of radioactivity incorporated into erythrocytes. Significantly more iron was absorbed from ferric trisglycinate administered in water (15.3%) than from

ferric trisglycinate administered in whole-maize porridge (2.3%). The percentages of the geometric mean of iron absorption in water were significantly different for the three sources of iron: ferrous ascorbate (32.5%) > ferric trisglycinate (15.3%) > ferrous glycinate (9.1%). Although in the first study significantly less iron was observed after administration of FeSO_4 in whole-maize porridge than after administration in water (as the reference dose, ferrous ascorbate), in this study no significant difference in iron absorption was reported from water or maize. On the basis of a highly significant negative correlation noted between iron absorption from any of the three sources of iron (ferrous ascorbate, ferrous glycinate, or ferric trisglycinate) and serum ferritin, the authors concluded that there is an effective down-regulation of iron absorption as iron reserves increase. They also concluded that the iron from ferrous glycinate does not exchange with the non-haem iron pool before intracellular incorporation; however, once inside the cell, its absorption is regulated by the same mechanisms as iron from FeSO_4 . Additionally, they concluded that ferrous glycinate is an effective and safe source of iron for use as a fortificant, particularly in foods rich in phytate.

The bioavailability of iron from an aqueous solution of ferrous glycinate (as Ferrochel® ferrous glycinate) was studied in a group of 14 healthy, non-pregnant, female volunteers aged 27–51 years (Olivares & Pizarro, 2001). The women were given 200 ml of an aqueous solution containing 111 kBq ^{55}Fe -Ferrochel® ferrous glycinate on day 1 and a similar volume of a reference dose solution containing 37 kBq ^{59}Fe -ascorbate on day 2, to correct for interindividual differences in iron status (both solutions providing 3 mg iron/day). Blood samples were obtained from each of the subjects 2 weeks later (on day 16 of the study) for measurement of erythrocyte radioactivity and iron status (i.e. haemoglobin, mean cell volume, free erythrocyte protoporphyrin, serum iron, total iron-binding capacity (TIBC) and serum ferritin). The bioavailability of iron from aqueous solutions of Ferrochel® ferrous glycinate and ferrous ascorbate was reported to be 34.6% and 29.9%, respectively. Upon standardization to 40% absorption of the reference dose of ferrous ascorbate, the corresponding absorption of iron from Ferrochel® ferrous glycinate was 46.3% (in a population with low iron stores). Nevertheless, these values were reported to be not significantly different for the two iron preparations. A significant inverse correlation was reported between the natural logarithm values of serum ferritin and iron absorption from Ferrochel® ferrous glycinate. According to the authors, this inverse relationship suggests regulation of iron absorption from Ferrochel® ferrous glycinate by iron stores (i.e. serum ferritin). This conclusion was further confirmed by a highly significant correlation between the natural logarithm values of iron absorption from aqueous solutions of ferrous ascorbate and Ferrochel® ferrous glycinate.

Olivares et al. (1997) studied the bioavailability of iron supplied as Ferrochel® ferrous glycinate administered in water or milk to groups of 14 healthy, non-pregnant female volunteers aged 33–51 years. In the first study, one group of women received 200 ml of an aqueous solution containing 15 mg/l of iron as 111 kBq ^{55}Fe -Ferrochel® ferrous glycinate on day 1, and 200 ml of a reference dose solution containing 15 mg/l of iron as 37 kBq ^{59}Fe -ascorbate on day 2 (both solutions providing approximately 3 mg iron/day). Venous blood samples were obtained from each of the subjects 2 weeks later (on day 16 of the study) for measurement of

erythrocyte radioactivity and iron status indicators (i.e. haemoglobin, mean cell volume, free erythrocyte protoporphyrin, serum iron, TIBC and serum ferritin). The women subsequently received 200 ml of whole cows' milk fortified with 15 mg/l of iron as 111 kBq ^{55}Fe -Ferrochel[®] ferrous glycinate (equivalent to 3 mg iron/day). Two weeks later (on day 31 of the study), venous blood samples were once again obtained for measurement of circulating erythrocyte radioactivity. The absorption of iron the two aqueous solutions of Ferrochel[®] ferrous glycinate (34.6%) and ferrous ascorbate (29.9%) was not significantly different between; however, a significantly lower iron bioavailability was reported from Ferrochel[®] ferrous glycinate administered in milk (8.3%) compared to that from Ferrochel[®] ferrous glycinate administered in water. Upon standardization of iron absorption to 40% of the reference dose of ferrous ascorbate, the corresponding percentages of iron absorption from Ferrochel[®] ferrous glycinate-fortified milk and water were 11.1% and 46.3%, respectively.

In a second study by Olivares et al. (1997), a group of 14 women were given 200 ml of an aqueous solution containing 15 mg/l of iron as 37 kBq ^{59}Fe -ascorbate on day 1, and 200 ml of whole cows' milk fortified with 15 mg/l of iron as 111 kBq ^{55}Fe -Ferrochel[®] ferrous glycinate plus 100 mg/l of ascorbic acid on day 2 (both solutions providing approximately 3 mg iron/day). For measurement of circulating radioactivity and determination of iron status, blood samples were obtained from the women on day 16 of the study. Fortification of whole cows' milk with Ferrochel[®] ferrous glycinate and ascorbic acid slightly increased iron absorption, from 8.3% to 10.7%, and from 11.1% to 15.4% when adjusted to 40% absorption of the reference dose, with the latter increase being statistically significant. To account for interindividual differences in iron status, the ratio of iron absorption from milk fortified with Ferrochel[®] ferrous glycinate to that from the reference dose solution (from study 1) was compared with the ratio of iron absorption from milk fortified with Ferrochel[®] ferrous glycinate plus ascorbic acid to that from the reference dose solution (from study 2). The ratio of iron absorption obtained from study 2 was significantly higher than that obtained from study 1. Significant inverse relationships were reported between serum ferritin and iron absorption from an aqueous solution of Ferrochel[®] ferrous glycinate or ferrous ascorbate. Additionally, the amount of iron absorbed from ferrous ascorbate was significantly and directly related to that absorbed from Ferrochel[®] ferrous glycinate in water or in milk (with ascorbic acid). According to the authors, these results suggested a controlled effect of the iron stores (i.e. serum ferritin) of the women on the absorption of iron from Ferrochel[®] ferrous glycinate. The amount of iron absorbed from milk fortified with Ferrochel[®] ferrous glycinate and ascorbic acid was not significantly correlated with serum ferritin or with the amount absorbed from the ferrous ascorbate reference dose solution. Therefore, the authors concluded that ascorbic acid had no significant effect on the absorption of iron from Ferrochel[®] ferrous glycinate-fortified milk.

A comparative study of the bioavailability of iron from Ferrochel[®] ferrous glycinate, Fe-EDTA, and FeSO_4 was conducted by Layrisse et al. (2000). A total of 74 healthy volunteers aged 15–50 years (18 male and 56 female) of low socio-economic status in Valencia, Venezuela, participated in five studies of absorption. Each person was allowed to participate in only one study, and participation was

determined by random selection. The number of participants and the meals administered in each study are presented in Table 1.

Blood samples were obtained from each person 15 days after they had eaten the appropriate meals (i.e. on days 16 and 30 of the study) for measurement of erythrocyte radioactivity and haemoglobin and serum ferritin concentrations. In study 1, significantly more iron was absorbed from foods fortified with Ferrochel[®] ferrous glycinate (8.4%) or FeEDTA (10.5%) than from unfortified (3.2%) or FeSO₄-fortified foods (4.7%). The mean iron absorption of 4/13 subjects who were iron-deficient in study 1 also was also reported to be significantly greater from foods fortified with Ferrochel[®] ferrous glycinate (13%) or FeEDTA (14%), than from foods fortified with FeSO₄ (6%). In study 2, significantly more iron was absorbed from foods fortified with Ferrochel[®] ferrous glycinate (10.8%) or FeEDTA (14.9%) than from unfortified (3.0%) or FeSO₄-fortified foods (5.3%). The mean iron absorption of 4/13 subjects who were iron-deficient in study 2 also was reported to be significantly higher from foods fortified with Ferrochel[®] ferrous glycinate (12%) or FeEDTA (15%), compared to that from foods fortified with FeSO₄ (7%). In study 3, significantly more iron was absorbed from Ferrochel[®] ferrous glycinate, than from FeSO₄ when they were administered together or in different meals. The percentages of iron absorbed from meals A (fortified with FeSO₄ and Ferrochel[®] ferrous glycinate), B (fortified with FeSO₄), and C (fortified with Ferrochel[®] ferrous glycinate) were reported to be 8.3%, 5.8%, and 9.7%, respectively; however, the difference in iron absorption between meals A and C was not statistically significant. Administration of 4 g of coffee or 1.6 g of tea with Ferrochel[®] ferrous glycinate-fortified corn meal (study 4) significantly decreased iron absorption from 7.5% to 3.9%; however, no significant change in iron absorption was observed when 2 g of coffee were taken with Ferrochel[®] ferrous glycinate-fortified corn meal (6.7%). In study 5, addition of phytase to corn flour meals fortified with FeSO₄ and Ferrochel[®] ferrous glycinate significantly increased iron absorption, from 5.1% to 10.1% and from 7.9% to 13.2%, respectively.

Allen et al. (1998) conducted a study involving the fortification of high-fat, unde-germed maize with iron as ⁵⁹FeSO₄ or ⁵⁵Fe-glycinate at 11 mg/g given to 10 fasted non-anaemic male volunteers (aged 19–30 years). The men ate a maize porridge meal containing 55.5 kBq ⁵⁹FeSO₄ at breakfast on day 1, and a similar meal containing 111 kBq ⁵⁵Fe-glycinate at breakfast on day 2. Blood samples were collected before the start of the study and 2 weeks after the second maize porridge meal had been eaten (i.e. on day 15 of the study). On day 15, the subjects were given maize porridge meals containing both 55.5 kBq ⁵⁹FeSO₄ and 111 kBq ⁵⁵Fe-glycinate. Two weeks later, blood samples were taken for measurement of erythrocyte radioactivity. Blood analysis indicated that four to seven times more iron was absorbed from ferrous glycinate than from FeSO₄. When the data were analysed by analysis of variance, the differences in absorption from the two sources of iron were highly significant. The mean values for iron absorption from ferrous glycinate-fortified meals were reported to be 8.68% and 6.95% when administered with and without FeSO₄, respectively, while the mean values for absorption from FeSO₄-fortified meals were 1.3% and 1.59% when administered with and without ferrous glycinate, respectively. Additionally, a negative correlation between ferritin and iron absorption was noted with both compounds, which the

Table 1. Composition of meals fortified with Ferrochel® ferrous glycinate, FeSO₄, or Fe-EDTA administered to volunteers participating in studies of iron absorption¹

Study No.	No. of subjects	Meal A	Meal B	Meal C	Meal D
1	13 (4 male 9 female)	Basal corn flour meal ² on the morning of day 1	Basal corn flour meal + 3mg of iron as ⁵⁵ FeSO ₄ on afternoon of day 1	Basal corn flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate on the morning of day 15	Basal corn flour meal + 3 mg of iron as ⁵⁵ Fe-EDTA on the afternoon of day 15
2	13 (1 male 12 female)	Basal wheat flour meal ³ on the morning of day 1	Basal wheat flour meal + 3mg of iron as ⁵⁵ FeSO ₄ on the afternoon of day 1	Basal wheat flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate on the morning of day 15	Basal wheat flour meal + 3mg of iron as ⁵⁵ Fe-EDTA on the afternoon of day 15
3	17 (all female)	Basal wheat flour meal + 3mg of iron as ⁵⁹ FeSO ₄ + 3mg of iron as ⁵⁵ Fe-Ferrochel® ferrous glycinate on day 1	Basal wheat flour meal + 3mg of iron as ⁵⁹ FeSO ₄ on day 15	Basal wheat flour meal + 3mg of iron as ⁵⁵ Fe-Ferrochel® ferrous glycinate on day 15	—
4	17 (8 male, 9 female)	Basal corn flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate on day 1	Basal corn flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate + 2g of a coffee beverage on day 1	Basal corn flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate + 4g of a coffee beverage on day 15	Basal corn flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate + a tea beverage (containing 1.6g of tea leaves) on day 15
5	14 (5 males, 9 female)	Basal corn flour meal + 3mg of iron as ⁵⁹ FeSO ₄ on day 1	Basal corn flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate on day 1	Basal corn flour meal + 3mg of iron as ⁵⁹ FeSO ₄ + 304U of phytase on day 15	Basal corn flour meal + 3mg of iron as ⁵⁹ Fe-Ferrochel® ferrous glycinate + 304U of phytase on day 15

¹ Table adapted from Layrisse et al. (2000)

² The basal corn flour meal consisted of 100g pre-cooked corn flour + 50g cheese + 10g margarine, with a basal iron content of 1.5mg

³ The basal wheat flour meal consisted of 100g white wheat flour + 50g cheese + 10g margarine, with a basal iron content of 1.6mg

authors reported to be suggestive of similar regulation of iron absorption by iron status for both iron sources.

Groups of infants aged 9 months participated in two studies of the bioavailability of iron from puréed vegetable meal and from whole-grain cereal fortified with [^{57}Fe]- or [^{58}Fe] ferrous glycinate or FeSO_4 (Fox et al., 1998). Solutions of the two iron compounds were used to fortify the meals, and in all cases, 0.83 mg of ascorbic acid was added per mg of iron present in [^{57}Fe]- or [^{58}Fe]- FeSO_4 solutions to reduce ferric iron to the ferrous form. In study 1, 22 infants (12 female and 10 male) with normal iron status (mean haemoglobin concentration, 114 g/l) were randomly assigned to two groups and given 150 g of commercial puréed vegetable weaning food (containing 1.62 mg of iron per serving) comprising parsnips, potatoes, cauliflower and milk, and fortified with FeSO_4 or ferrous glycinate at 1.4 mg per serving as. One group received puréed vegetable meals fortified with [^{57}Fe]- FeSO_4 or [^{58}Fe]-glycinate, while the other group received similar meals fortified with [^{58}Fe]- FeSO_4 or [^{57}Fe]-glycinate, both on alternating days for 8 days. Fourteen days after the last test meal, all the infants were subjected to heel-prick blood sample testing, iron isotope ratio and body weight were measured, and blood volume was estimated. The mean per cent incorporation of iron from FeSO_4 (9.1%) and ferrous glycinate (9.8%) into haemoglobin was reported to be not significantly different. In a second study, 24 infants (8 female and 16 male) with normal iron status (mean haemoglobin concentration, 118 g/l) were randomly divided into two groups and given 150 g of a puréed vegetable meal (as in study 1) or 20 g of a dry, commercial, whole-grain cereal food (containing 1.57 mg of iron per serving) made from wheat, rye, oats, rice, wheat bran, and 120 g milk, and fortified with FeSO_4 or ferrous glycinate at 1.4 mg iron per serving as. Group 1 received [^{57}Fe]- or [^{58}Fe]- FeSO_4 , with the puréed vegetable meal or whole-grain cereal meal, administered alternately. Group 2 received [^{57}Fe]- or [^{58}Fe]-glycinate alternately with the same meals as group 1. Heel-prick blood samples were obtained from the infants 14 days after the last test meal, and body weights were measured and total circulating blood volume estimated. As in study 1, the mean incorporation of iron from FeSO_4 (9.1%) and from ferrous glycinate (9.8%) into haemoglobin was not significantly different when these substances were administered to infants in a puréed vegetable meal. Lower iron bioavailability was, however, reported for FeSO_4 (3.8%) and ferrous glycinate (5.2%) administered in a whole-grain cereal meal, although this difference was not statistically significant. The authors attributed the significantly lower iron bioavailability from the whole-grain cereal meal to its significantly higher phytic acid content (77.5%) compared to that of the puréed vegetable meal (22.1%). According to Fox et al. (1998), the inhibiting effect of phytic acid on the bioavailability of iron from ferrous glycinate suggested dissociation of iron from the iron–glycine complex and its subsequent incorporation into the common non-haem iron pool in the gastrointestinal tract.

2.1.3 Biotransformation

Both the iron and glycine components of ferrous glycinate are metabolized via normal metabolic processes, without the generation of exogenous chemicals as a result of biotransformation.1

2.1.4 Influence of ferrous glycinate on the chemistry of nutrients

As previously noted, the chemical structure of ferrous glycinate confers protection from oxidation and other chemical reactions with compounds in the food matrix. The addition of ferrous glycinate to multivitamin preparations has been reported not to affect the stability of vitamins.

Iron Metalosate[®], an animal feed supplement consisting of iron amino acid chelates containing ferrous glycinate, added to provide a concentration of iron of 5 mg/ml to a solution of vitamin A (20 000 IU/ml), had no effect on the level of vitamin A over 324 days (Marchetti et al., 2000). An accelerated degradation of vitamin A activity was, however, observed in the presence of 5 mg/ml of ferrous chloride.

The stability of vitamins and minerals was assayed in a 17-month study of a preparation in which several metal amino acid chelates and a multivitamin preparation containing vitamins A, C, D and E, and the vitamin B series were integrated into a powdered blend and subsequently encapsulated (Ashmead & Ashmead, 1995). Each capsule was formulated to contain Chelazome[®] metal amino acid chelates of copper, manganese, and zinc at concentrations of 0.45–0.55 mg, 0.86–1.05 mg, and 1.37–1.67 mg, respectively. Additionally, 36–44 µg of chromium (as the chelate product Chromium Chelavite[®]) and 1.8–2.2 mg of iron supplied as Ferrochel[®] ferrous glycinate were incorporated into each capsule. The stability of these chelated products was demonstrated by a lack of interaction between the metal amino acid chelates and any of the components of the capsules, including the vitamins, which were reported to retain levels within the specifications of the United States Pharmacopeia for the duration of the study.

2.2 Toxicological studies

2.2.1 Acute toxicity

The oral LD₅₀ for ferrous glycinate (as Ferrochel[®] ferrous glycinate) was determined to be 2800 mg/kg bw in male and female rats, which corresponds to an iron concentration of approximately 560 mg/kg bw (95% CI, 399–786 mg/kg bw) (Kukulinski, 1993; Jeppsen & Borzelleca, 1999). Signs of toxicity observed in the rats included hunched posture, hypoactivity, hypothermia, prostration, poor coordination, and loose stools.

2.2.2 Short-term toxicity

A range-finding study was conducted in CD Sprague-Dawley rats. Groups of three male and three female rats received 0, 300, or 500 mg/kg bw of ferrous glycinate (as Ferrochel[®] ferrous glycinate, containing 20.34% iron and 55% chelated glycine by weight) orally by gavage for 14 days. These treatments corresponded to doses of iron of approximately 0, 60, and 100 mg/kg bw per day. The rats were observed twice daily for signs of morbidity or mortality, and body weights and food consumption were measured weekly. The animals were killed at the end of the treatment period and a complete gross pathological examination was conducted. No deaths occurred during the study. With the exception of a red focus in the glandular portion of the stomach and thick black material in the caecum of female

receiving the highest dose (500 mg/kg bw), no other clinical findings were reported in any of the rats treated with Ferrochel® ferrous glycinate (Jeppsen & Borzelleca, 1999).

On the basis of the results of the 14-day range-finding study, groups of CD Sprague-Dawley rats (20 of each sex per group) were fed ferrous glycinate (as Ferrochel® ferrous glycinate) in the diet at a concentration of 0, 100, 250, or 500 mg/kg bw per day for 13 weeks (Jeppsen & Borzelleca, 1999; Mandella, 2000). The actual daily intake of Ferrochel® ferrous glycinate was reported to be 0, 99.6, 249, and 497 mg/kg bw for males, and 0, 99.4, 247.3, and 499.4 mg/kg bw for females. This study was conducted in compliance with the United States Food and Drug Administration regulations for good laboratory practices. The animals were given access to water *ad libitum*. They were observed twice daily for signs of pharmacological or toxicological effects, morbidity and mortality, and a thorough physical examination was performed three times before initiation of the study, as well as weekly thereafter. Food consumption was measured twice and body weights were measured three times before the start of the study, and weekly thereafter. Ophthalmoscopic examinations were performed on each animal before initiation of the study and before necropsy at the end of 13 weeks of treatment. Blood samples were drawn for determination of haematological and clinical chemical parameters. The haematological parameters evaluated included haemoglobin concentration, erythrocyte volume fraction, erythrocyte count and morphology, reticulocyte count, platelet count, mean corpuscular volume, mean corpuscular haemoglobin concentration, prothrombin time, activated partial thromboplastin time, and total and differential leukocyte counts. The clinical chemistry parameters evaluated included the activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and γ -glutamyl transferase, the concentration of blood urea nitrogen, fasting glucose, total protein, albumin, globulin (calculated), albumin:globulin ratio (calculated), creatinine, total bilirubin, sodium, potassium, chloride, calcium and inorganic phosphorus. Upon gross necropsy of all animals at the end of 13 weeks, the adrenal glands, brain, kidneys, testes with epididymides, liver, ovaries, thymus, spleen and stomach were removed and weighed. A total of 60 samples of tissues and organs (including the lungs, liver, and kidney) from animals in the control groups and groups receiving the highest dose (500 mg/kg bw per day) were preserved for histopathological examination, and on the understanding that any tissue found to be abnormal in animals in the group receiving the high dose would also be examined in animals receiving the two lower doses. No deaths occurred during the study, and no compound-related, dose-dependent adverse effects on cageside observations, the results of physical and ophthalmoscopic examinations, body-weight gain, food consumption, haematological and clinical chemistry parameters, and absolute and relative organ weights were reported. Male rats at all doses and females at the highest dose had significantly increased food consumption at several intervals throughout the study, but this was not considered to be toxicologically significant. The mean hepatic non-haem iron concentration was increased by approximately 1.6-fold in males at the two higher doses, and by about 1.4-fold in females at the highest dose of Ferrochel® ferrous glycinate, compared with controls. These increases were not linearly related to dose, however, indicating the presence of a physiological control

on the absorption and distribution of the iron from Ferrochel® ferrous glycinate. No significant differences in mean haemoglobin concentration or erythrocyte volume fraction were reported. Slight but statistically significant increases in mean corpuscular volume and haemoglobin were reported in males receiving the highest dose when compared to controls, but these values remained within the normal range, and were therefore not considered to be toxicologically significant. Males receiving the two lower doses had significantly lower concentrations of potassium than controls, and males receiving the intermediate dose also had decreased activity of aspartate aminotransferase; however, these effects were not related to dose and were considered not to be toxicologically significant. Two females receiving the intermediate dose had significantly increased absolute and relative spleen weights when compared with controls, but these effects were not considered to be toxicologically significant owing to the absence of a dose–response relationship. Histopathological examination revealed no biologically or statistically significant, dose-dependent, macroscopic or microscopic findings that could be attributed to treatment with Ferrochel® ferrous glycinate. The NOAEL for Ferrochel® ferrous glycinate was 500 mg/kg bw per day, corresponding to a dose of iron of approximately 100 mg/kg bw per day.

2.2.3 Long-term studies of toxicity and carcinogenicity

No information was available.

2.2.4 Genotoxicity

No information was available.

2.2.5 Reproductive toxicity

No information was available.

2.2.6 Observations in humans

Ferrous glycinate (or Ferrochel® ferrous glycinate) has been used in field trials as a source of iron for food enrichment and fortification purposes in various countries, including Brazil (Queiroz & Torres, 1995; Fisberg et al., 1995; Gualandro & Name, 1996; Ashmead et al., 1997; Iost et al., 1998; Giorgini et al., 2001; Szarfarc et al., 2001), Guatemala (Pineda et al., 1994; Pineda & Ashmead, 2001), New Zealand (Heath et al., 2001), Saudi Arabia (Osman & al-Othaimen, 2002) and the United Republic of Tanzania (Latham et al., 2001). These studies have shown that the use of ferrous glycinate (or Ferrochel® ferrous glycinate) as a dietary supplement or iron fortificant in foods is efficacious in reducing the prevalence of iron deficiency and iron deficiency anaemia in humans.

(a) Ferrous glycinate supplementation

Coplin et al. (1991) assessed the tolerability of daily doses of 50 mg of iron supplied as Ferrochel® ferrous glycinate or FeSO₄ in a randomized, double-blind,

crossover study of 40 pre-menopausal, non-pregnant women (aged 18–40 years) with normal iron status. The women were given a single capsule containing 50 mg of iron each morning before breakfast for 14 days. The severity and prevalence of eight symptoms (i.e. abdominal pain, bloating, constipation, diarrhoea, nausea, vomiting, headache, and fatigue), as well as any other self-reported symptoms, were evaluated by the women daily, on a scale of 0 (none present) to 3 (severe). A total of 38 women finished the 28-day study period, two women having withdrawn for reasons other than intolerance to the iron preparations. No severe scores were reported, and all the side-effects were evaluated as being mild to moderate. The incidence of side-effects did not increase from week 1 to week 2 of the treatment period, indicating that the effects were not cumulative. Supplementation with Ferrochel® ferrous glycinate or FeSO₄ produced no significant difference in the frequency of side-effects, with weekly averages of 9.4 and 9.3 occurrences, respectively. Adverse effects while receiving Ferrochel® ferrous glycinate and FeSO₄ supplementation were reported by a total of 25/38 (66%) and 26/38 (68%) participants, respectively. Of these, gastrointestinal side-effects were reported in 24%, 34%, and 32% of those receiving supplements of Ferrochel® ferrous glycinate, FeSO₄, and both sources of iron, respectively. Of the 28 women with total scores for gastrointestinal side-effects of ≥5, 29%, 50%, and 21% of women had received supplements of Ferrochel® ferrous glycinate, FeSO₄, and both sources of iron, respectively. These differences were not statistically significant. A rank sum analysis of symptom scores was performed for each subject, whereby the symptom score for the FeSO₄ preparation was subtracted from the score for the Ferrochel® ferrous glycinate preparation, and the differences were subsequently ranked and summed. On the basis of the results of this analysis, the authors concluded that there was no significant difference in tolerance to the two iron preparations. In terms of total scores for gastrointestinal side-effects (especially bloating, constipation and nausea), however, a better tolerance for the Ferrochel® ferrous glycinate than to the FeSO₄ preparation was noted. Significantly more women preferred the Ferrochel® ferrous glycinate preparation (61%) to the FeSO₄ preparation (29%). The authors predicted that a significant difference in the frequency of side-effects between the two preparations would have been observed with a larger sample size of 120 subjects (Coplin et al., 1991).

Forty-two males and 58 females (aged 10–19 years) from the lowlands of Guatemala with a mean haemoglobin concentration of 11.23 g/dl (indicative of anaemia) were assigned to one of four groups (21–27 individuals per group). The participants received tablets containing 30 mg of iron supplied as Ferrochel® ferrous glycinate once (group 1), twice (group 2), or four times (group 3) daily for a period of 4 weeks, thus receiving a total dose of 30, 60, or 120 mg iron/day, respectively. Another group (group 4) received a total daily dose of 120 mg of iron supplied as FeSO₄, administered as four tablets containing 30 mg iron per tablet for a period of 4 weeks. All of the subjects were also provided with 250 µg of folic acid per total daily dose of iron. The tablets were taken at breakfast or, in the case of persons receiving 120 mg iron per day (groups 3 and 4), two of the tablets were taken at breakfast while the other two tablets were taken at supper. Blood samples were obtained from each person before the start of the study, and at the end of treatment, to measure basal and post-treatment plasma concentrations of haemo-

globin and ferritin. No significant differences among the groups in age, sex, or pre-treatment haemoglobin concentrations were reported. At the end of the 4-week treatment period, a significant increase in mean haemoglobin concentration (2.6 g/dl) over the basal value was reported in all four treatment groups; however, this increase was reported not to be significantly different between groups. The plasma ferritin concentrations of individuals in groups 2, 3, and 4 (dosed with 60 or 120 mg iron/day as Ferrochel[®] or FeSO₄) were significantly increased over pre-treatment values, but no significant difference was found between groups. The plasma ferritin concentrations of individuals in group 1 were lower than basal values at the end of the 4 weeks of treatment. Gastrointestinal "upsets" were reported on days 15 and 30 of the study in 2/21 (9.5%), 4/26 (15.4%), and 9/27 (33.3%) of the persons in groups 2, 3 and 4, respectively. No such effects were reported by individuals in group 1. The authors concluded that daily dosing with 60 mg of iron, supplied as Ferrochel[®] ferrous glycinate, is effective in the treatment of iron deficiency anaemia, and has almost no gastric side-effects (Pineda et al., 1994).

Forty infants (aged 6–36 months) with iron deficiency anaemia (haemoglobin concentration of <11 g/dl), in the paediatric department of the San Juan de Dios General Hospital in Guatemala City, Guatemala, were paired for age, sex, weight and haemoglobin concentration, and randomly assigned to one of two groups (20 infants per group) in a double-blind controlled study. The children received a single daily dose of 250 µg folic acid and a dose of iron of 5 mg/kg bw in the form of a syrup containing 30 mg/ml as FeSO₄ or Ferrochel[®] ferrous glycinate for 28 days. Blood samples were obtained from each child before and after the treatment for determination of haemoglobin and ferritin concentrations. Haemoglobin concentrations were significantly increased by the end of the treatment period in groups receiving supplements of FeSO₄ or Ferrochel[®] ferrous glycinate over baseline values, but did not differ between groups. The change in haemoglobin concentrations was reported to be inversely proportional to basal levels, regardless of the iron compound administered. Mean plasma ferritin concentrations were increased over baseline values in both treatment groups, but the increase was statistically significant only in the group given Ferrochel[®] ferrous glycinate (Pineda & Ashmead, 2001).

A 16-week, double-blind, placebo-controlled intervention study was conducted in 75 women (aged 18–40 years) with mild iron deficiency (i.e. serum ferritin concentration, <20 µg/l; haemoglobin concentration, ≥120 g/l) and residents of Dunedin, New Zealand. The participants were randomly assigned to one of three groups receiving either a placebo tablet containing maltodextrin and regular diet (placebo group), a Ferrochel[®] ferrous glycinate tablet and regular diet (supplement group), or a placebo tablet and increased dietary iron intake (diet group). Laboratory analyses indicated that each tablet of Ferrochel[®] ferrous glycinate and placebo contained approximately 47 mg and 0.7 µg of iron, respectively, and participants took a single daily dose of the appropriate tablet with a meal for 16 weeks. Participants in the diet group were provided with dietary advice by New Zealand-registered dieticians in order to increase their dietary iron intake and the bioavailability of that iron (e.g. use of recipe books and cast-iron frying pans, and consumption of foods and beverages providing at least 50 mg of vitamin C per meal). Fasting blood samples were obtained from each woman once a month for determination

of concentrations of haemoglobin, serum ferritin, serum transferrin receptor, and C-reactive protein. The average monthly dietary intake of haem, non-haem, and total iron was estimated from a food frequency questionnaire filled out by the women at study recruitment and during weeks 4, 8, and 16 of the study. Data on blood loss due to menstruation, blood donation and nosebleeds were collected from pre-tested questionnaires. The study was completed by 57 women (19, 16, and 22 women in the placebo, supplement, and diet groups, respectively). Eight of the women withdrew from the study because of personal reasons; while the data from another 10 women were excluded because they had anaemia, had donated blood, or were pregnant. The number of withdrawals and exclusions was not significantly different between groups. Additionally, no significant differences in the frequency and severity of adverse effects (i.e. abdominal discomfort, bloating, constipation, diarrhoea, nausea, vomiting, headache and fatigue) were reported between the placebo and supplement or diet groups (excluding symptoms occurring immediately before or during menses). There were no significant differences in the concentrations of haemoglobin, serum ferritin, or serum transferrin receptor, or in blood loss variations (e.g. menstruation and nosebleeds) reported between groups. A 97% compliance rate was reported for the group receiving the supplement. No significant differences in the mean dietary intake of haem, non-haem, and total iron were reported between the placebo and supplement groups; however, these parameters were significantly increased (haem iron, by 1.9 mg/day) in the diet group when compared with the placebo group. The total iron intakes of the three groups were reported not to be significantly different from each other. After 16 weeks, the serum ferritin concentrations in the diet and supplement groups were increased by 26% and 59%, respectively, over those in the placebo group; however, this increase was statistically significant only in the group receiving the supplement. The concentrations of haemoglobin at 16 weeks were not significantly different from baseline values in both the diet and supplement groups. The concentration of serum transferrin receptor and ratio of serum transferrin receptor: serum ferritin were significantly decreased in the supplement group compared to baseline values. According to the authors, the simultaneous decrease in serum transferrin receptor concentration and increase in serum ferritin concentration in the supplement group suggested concurrent replenishment of the functional tissue and storage iron compartments. No significant changes in serum transferrin receptor concentration or the ratio of serum transferrin receptor: serum ferritin compared with baseline values were reported in the diet group. The authors concluded that improvements in iron status could be achieved to a greater degree by iron supplementation than with dietary change, owing, in part, to the difficulties associated with embarking on and sustaining a dietary regimen requiring increased consumption of flesh foods (Heath et al., 2001).

A group of 145 pregnant female volunteers in Santo André, Brazil were given FeSO₄ at 200 mg/day or Ferrochel® ferrous glycinate at 75 mg/day, providing iron at 40 and 15 mg/day, respectively, from week <20 of pregnancy until parturition. A total of 74 women received FeSO₄ supplements, while 71 women received Ferrochel® ferrous glycinate supplements. Blood samples were obtained from each of the subjects at the time of enrollment in the study between weeks 20 and 29 of pregnancy, and at 30 or more weeks of pregnancy. Haematological parameters

evaluated included the concentrations of haemoglobin and serum ferritin, and the percentage saturation of transferrin was calculated from the serum concentrations of iron, TIBC and ferritin. Compliance with supplement intake was reported to be significantly greater among women given Ferrochel[®] ferrous glycinate, with 52/71 (73%) and 26/74 (35%) of the women taking Ferrochel[®] ferrous glycinate and FeSO₄ supplements, respectively, for ≥ 13 weeks. The taste of the iron supplement was the main reason for non-compliance by the women being given FeSO₄. None of the women receiving Ferrochel[®] ferrous glycinate reported that it had a bad taste. Five women reported nausea, vomiting or diarrhoea as the reason for their non-compliance when taking iron as FeSO₄, while two women reported these symptoms while taking iron as Ferrochel[®] ferrous glycinate. The authors attributed the greater compliance with Ferrochel[®] ferrous glycinate supplementation to the fact that the supplement could be ingested with meals, thus having fewer adverse gastric effects. There was a trend towards decreasing concentrations of haemoglobin and serum ferritin in the women as pregnancy progressed; however, the decreases in serum ferritin and percent transferrin saturation were lower in the group taking Ferrochel[®] ferrous glycinate than in the group taking FeSO₄. The statistical significance of these decreases was not reported by the authors. Much information was missing, as only about 33% of the women who were originally enrolled in the study provided blood samples on all three sampling occasions. This problem was compounded by sample losses in the laboratory. When the authors analysed the results for women who had provided blood on all three occasions, significantly higher concentrations of haemoglobin and serum ferritin, and significantly higher percent transferrin saturation were reported for the women receiving Ferrochel[®] ferrous glycinate supplements as compared with women receiving FeSO₄. Nevertheless, there was a large discrepancy in the sample sizes used in this analysis, with ranges of 7–14 and 17–40 in groups given Ferrochel[®] ferrous glycinate and FeSO₄, respectively. Supplementation with Ferrochel[®] ferrous glycinate in compliant women at ≥ 30 weeks of pregnancy was reported to result in lower incidences of iron deficiency (31%) and iron deficiency anaemia (0%) than supplementation with FeSO₄ (incidence of iron deficiency, 54.5%; iron deficiency anaemia, 11%) (Szarfarc et al., 2001).

(b) *Ferrous glycinate-fortified foods*

Ashmead et al. (1997) gave groups of seven persons (age and sex not reported; matched for age, sex and initial haemoglobin status) with iron at a dose of 22.5 mg/day as Ferrochel[®] ferrous glycinate or at 45 mg iron/day as FeSO₄ for 28 days. Except for two infants who received iron in 100 ml of milk, the participants ate 20 g of a sweetened peanut butter bar (*paçoca*, a common snack in Brazil) fortified with iron from FeSO₄ or Ferrochel[®] ferrous glycinate. Baseline and post-treatment concentrations of haemoglobin and serum ferritin were determined for each participant. At the end of 28 days of fortification, both groups had significantly higher haemoglobin and serum ferritin concentrations than at baseline, but these increases were not significantly different between groups. According to the authors, the bioavailability of iron from Ferrochel[®] ferrous glycinate was at least twice that from FeSO₄, since only half as much iron was given to the group receiving Ferrochel[®] ferrous glycinate, with results equivalent to that of the group receiving

FeSO₄. Additionally, owing to the significant correlation between body iron status and the magnitude of iron absorption observed with both iron compounds, the authors concluded that there is little danger of iron overloading or subsequent toxicity in people with normal iron metabolism who eat foods fortified with either FeSO₄ or Ferrochel® ferrous glycinate. Furthermore, the authors considered that the lower intake of iron from Ferrochel® ferrous glycinate provides a greater margin of safety for food fortification (Ashmead et al., 1997).

A similar study was conducted in 434 people (9% of whom were aged 1–3 years and 16% of whom were aged >15 years) given 20 g of sweetened peanut butter fortified with iron at 45 mg/day as FeSO₄ or 23 mg/day as ferrous glycinate for an unspecified duration. Baseline and post-treatment concentrations of haemoglobin and serum ferritin were measured for each person. Both compounds were reported to be well tolerated. The mean haemoglobin values for the group receiving FeSO₄ increased from 10.35 to 11.94 g/dl, while those of the ferrous glycinate group increased from 10.61 to 12.13 g/dl. Serum ferritin concentrations were also increased in the group receiving FeSO₄, from 6.87 to 22.07 µg/l, while those in the group receiving ferrous glycinate increased from 5.38 to 14.35 µg/l; however, the authors cautioned that some of the serum ferritin values might have been artificially high, owing to infections or disease. The increases in haemoglobin and serum ferritin concentrations in both groups were reported to be statistically significant, but the differences in increases between groups were not. The authors concluded that 23 mg of iron as ferrous glycinate was as effective as 45 mg of iron as FeSO₄ in correcting iron deficiency anaemia (Gualandro & Name, 1996).

Milk fortified with Ferrochel® ferrous glycinate at a concentration of 30 mg/l was given daily to 131 children aged 6–14 years in Riyadh, Saudi Arabia, for 3 months. Milk of three different flavours (plain, strawberry, and chocolate) was served and drunk three times daily with each meal. Social workers supervised the milk distribution and consumption. The total daily iron consumption was estimated to be 6 mg per child. Blood samples were obtained from each child at study initiation and termination. The milk fortified with Ferrochel® ferrous glycinate was reported to be well accepted by the children, the order of flavour preferences being: chocolate > strawberry > plain and was also well tolerated by the children, with no reports of gastrointestinal problems during the course of the study. The authors reported no clinical indications of iron overload in any of the children. The prevalence of haemoglobin concentrations of <12 g/dl dropped significantly, from 25.3% to 5.0% and from 23.0% to 8.6%, for boys and girls, respectively ($p < 0.0001$). The authors concluded that fortification of milk with Ferrochel® ferrous glycinate was an effective way of increasing the haemoglobin concentrations of children with low or close to normal haemoglobin levels (Osman & Al-Othaimen, 2002).

Commercially prepared *petit suisse* cheese was fortified with ferrous glycinate to provide iron at 2 mg/90 g of cheese (approximately equivalent to 22.2 mg/kg of cheese) and given to 81 children (aged 2–6 years) of low socioeconomic status in São Paulo, Brazil, for 90 days. The concentrations of haemoglobin, ferritin, plasma zinc and copper, and anthropometric parameters were measured at the beginning and end of the study period. The children were reported to have an average daily consumption of 90 g of cheese, approximately equivalent to a daily iron intake of

2 mg. The cheese was adequately accepted and well tolerated by the children, as no product-related adverse effects were reported throughout the duration of the study. Mean pre-treatment values of 12.27 g/dl of haemoglobin and erythrocyte volume fraction of 0.38 were reported in 10.5% of the children, while 60% of children had a mean serum ferritin concentrations of 15.7 ng/ml. At the end of 90 days of treatment, 6.3% of the children had iron deficiency anaemia and 20.5% had levels of ferritin that were below normal. The improvements in the average values of haemoglobin and erythrocyte volume fraction were not significantly different from pre-treatment values; however, the increase in average serum ferritin concentration from 15.69 to 24.68 ng/ml was statistically significant. The average serum zinc concentration was reported to have decreased substantially throughout the study, but remained within the normal range. There were no changes in the overall consumption of food by the children, although the ratios of weight:age and weight:height increased as the study progressed; the authors attributed this to the reduction of iron deficiency parameters in the children. The authors concluded that ferrous glycinate is effective in increasing haemoglobin concentrations and replenishing iron reserves in children (Fisberg et al., 1995).

Sweet rolls prepared from ferrous glycinate-fortified flour were provided twice daily, 5 days a week (providing iron at approximately 4 mg per day) for 6 months to 89 children aged 1–6 years of low socioeconomic status in São Paulo, Brazil. Haematological (i.e. concentrations of haemoglobin and serum ferritin) and anthropometric parameters (i.e. body weight and height) were measured before the start and at the end of the intervention. At the end of the 6-month period, the mean ratios of body weight:age and height:age were significantly greater than the pre-intervention values. The haemoglobin concentration increased significantly (from 11.5 to 12.6 g/dl), the prevalence of iron deficiency anaemia (concentration of haemoglobin, <11 g/dl) decreased significantly (from 28% to 9%), and the prevalence of low iron stores decreased significantly (from 62% to 25%) in all children. The average increase in haemoglobin concentrations (1.10 g/dl) was reported to be significant for all children, with a greater mean increase (1.42 g/dl) in anaemic children than in those with a pre-intervention haemoglobin concentration of ≥ 11 g/dl (mean increase of 0.96 g/dl). Significant increases (mean increase, 8.90 μ g/l) were also reported in the mean serum ferritin concentrations, particularly in children with depleted iron stores (mean increase, 13.03 μ g/l). According to the authors, the greater increase in serum ferritin concentrations observed in children with lower basal concentrations of ferritin (as compared with the increases in subjects with concentrations closer to normal) is consistent with the regulation of iron absorption from ferrous glycinate by iron stores (Giorgini et al., 2001).

A randomized, double-blind, placebo-controlled study was conducted in 775 children (aged 6–12 years) in the United Republic of Tanzania. For 6 months, each child received a sachet containing 25 g of a placebo or fortified powdered orange drink, which provided iron as ferrous glycinate at 0 or 5.4 mg/day. Each sachet also contained 1750 IU vitamin A, 45 μ g iodine, 5.25 mg zinc, 72 mg ascorbic acid, 0.6 mg riboflavin, 0.14 mg folic acid, 3 μ g vitamin B₁₂, 0.7 mg vitamin B₆ and 10.5 mg vitamin E. Anthropometric parameters (weight, height, body mass index (BMI)), and vitamin A and iron status (i.e. haemoglobin, erythrocyte volume fraction, zinc protoporphyrin, and serum ferritin) were measured at the beginning and end of the

study. The pre-treatment levels of serum retinol and measures of iron status and the anthropometric parameters were not significantly different between groups given the placebo and the fortified orange drink. The mean haemoglobin concentration for the group given the fortified orange drink significantly increased, from 9.3 to 10.6 g/dl, during the 6-month intervention period when compared with that for the placebo group, in whom the mean haemoglobin concentration increased by only 0.02 g/dl. There also was a significant increase (16 µg/l) in mean serum ferritin concentrations in the group given the fortified drink when compared with that (2 µg/l) noted in the group receiving the placebo at the end of the intervention. The group given the fortified drink also had a significantly lower incidence of vitamin A deficiency after 6 months than the group treated with the placebo; the number of children with a serum retinol concentration of <20 µg/dl (indicating vitamin A deficiency) was decreased by 50% in the former group. Highly significant increases in body-weight gain, height gain, and BMI were also found in the group receiving the drink fortified with micronutrients when compared with the group receiving the placebo (Latham et al., 2001).

One litre of milk fortified with Ferrochel® ferrous glycinate at 15 mg/l (containing a concentration of iron of 3 mg/l) was given daily for 7.3 months to 185 infants and young children (aged 6–24 months) in Tupã, Brazil. According to the WHO guidelines for identification of iron deficiency anaemia (haemoglobin concentration: severe anaemia, <9.5 g/dl; less severe, 9.5–11.0 g/dl; normal, >11.0 g/dl), severe iron deficiency anaemia was diagnosed in 54% of the children, 33% were classified as having less severe iron deficiency anaemia, and 13% were considered to have normal haemoglobin concentrations. At the conclusion of the study, the children who remained severely anaemic were given higher doses of iron. The average daily consumption of Ferrochel® ferrous glycinate-fortified milk was estimated to be 750 ml, providing iron at approximately 2.1 mg/day. Finger-prick blood samples were obtained from each child before the start of the study, 4.4 months into the study (i.e. at a mean of 133 days), and at the end (i.e. at a mean of 222 days) in order to monitor any changes in haemoglobin concentration. The mean haemoglobin concentrations for all children were 9.3, 10.5, and 11.2 g/dl at 0, mean 133 days, and mean 222 days of the study, respectively. At a mean of 222 days of the study, significantly increased haemoglobin concentrations were found in children who were initially diagnosed with severe or less severe anaemia, but no significant changes were reported in those who initially had normal haemoglobin concentrations. The authors concluded that fortification of commercial milk with Ferrochel® ferrous glycinate is an effective dietary intervention for the treatment of iron deficiency and iron deficiency anaemia in children (lost et al., 1998).

In a study in Angaluba, Brazil, whole milk was fortified with iron as ferrous glycinate at a concentration of 3 mg/l, and 1 litre was given daily to 269 preschool children for 12 months. The prevalence of anaemia was significantly reduced, from 62.3% at the beginning of the study to 26.4% at the end. Specifically, the prevalence of children with severe anaemia (haemoglobin concentration, ≤9.5 g/dl) was reduced from 20.4% to 5.2%, while the prevalence of those with moderate anaemia (haemoglobin concentration, 9.5–10.9 g/dl) was decreased from 31.1% to 16.6%, and the prevalence of those with normal iron status (haemoglobin concentration,

≥11 g/dl) increased from 31.2% to 60.9%. The authors concluded that fortification of milk with ferrous glycinate was an effective way of reducing, and in some cases eliminating, iron deficiency anaemia in children (Queiroz & Torres, 1995).

3. COMMENTS

The Committee noted that ferrous glycinate is absorbed by the mucosal cells of the intestine, and is subsequently dissociated into its iron and glycine components within the intestinal mucosa. The available studies indicate that the absorption of iron from ferrous glycinate is regulated physiologically according to the body's iron status, in a manner similar to other non-haem iron compounds. The bioavailability of iron from ferrous glycinate is comparable to that of Fe-EDTA (evaluated by the Committee at its forty-first and fifty-third meetings; Annex 1, references 107 and 143) and is generally greater than that of FeSO₄. As is the case with other non-haem iron compounds, the nature of the food matrix may affect the bioavailability of the iron from ferrous glycinate.

In consideration of the potential for overuse of this product, the Committee noted the results of studies of dietary supplementation and fortification at doses of up to 60 mg iron per day, which confirmed the efficacy of ferrous glycinate in correcting iron status in persons with iron deficiency, while showing no gastric side-effects. In iron-sufficient individuals, including children, iron absorption from ferrous glycinate is down-regulated according to iron status, and haemoglobin and serum ferritin concentrations are not significantly increased relative to pre-treatment or normal-range values at doses of up to 23 mg iron per day. The Committee therefore concluded that there was no evidence that the administration of iron in the form of ferrous glycinate would result in increased body stores of iron after the nutritional requirement for iron had been satisfied.

The Committee reviewed a 90-day study of toxicity in rats fed diets containing ferrous glycinate. Despite the fact that a slight increase in iron deposition in the liver of rats of each sex occurred at high doses, no compound-related toxicological effects were noted at doses of 100, 250 or 500 mg/kg bw per day. The NOEL of ferrous glycinate was 500 mg/kg bw per day, corresponding to a concentration of iron of 100 mg/kg bw per day. This NOEL is 125-fold the provisional maximum tolerable daily intake of 0.8 mg/kg bw of iron from all sources.

4. EVALUATION

On the basis of the available data on bioavailability, metabolism, and toxicity, and the studies in humans, the Committee concluded that ferrous glycinate is suitable for use as a source of iron for supplementation and fortification, provided that the total intake of iron does not exceed the provisional maximum tolerable daily intake of 0.8 mg/kg bw.

Products, including ferrous glycinate, that are intended to provide a source of additional iron should not be consumed by individuals with any type of iron storage disease, except under medical supervision.

The Committee did not receive information on estimated intakes of ferrous glycinate, either from its use in food or any possible use as an iron supplement. Information on levels of fortification in food, provided by the sponsor, suggest that intakes approaching the provisional maximum tolerable daily intake could not be attained unless extremely large amounts of foodstuffs fortified at the suggested levels were eaten.

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SODIUM DICHLOROISOCYANURATE

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Explanation	487
Biological data	488
Biochemical aspects: absorption, distribution and excretion	488
Sodium dichloroisocyanurate.....	488
Sodium cyanurate.....	488
Toxicological studies	489
Acute toxicity.....	490
Short-term studies of toxicity.....	490
Long-term studies of toxicity and carcinogenicity	492
Reproductive toxicity.....	493
Genotoxicity	495
Special studies: Immunotoxicity	495
Observations in humans	495
Dietary intake.....	495
Comments	496
Evaluation	497
References	499

1. EXPLANATION

Sodium dichloroisocyanurate is the sodium salt of a chlorinated hydroxytriazine and is used as a source of free available chlorine (in the form of hypochlorous acid, HOCl) for the disinfection of drinking-water. Sodium dichloroisocyanurate can be manufactured either as the anhydrous salt or as the dihydrate. It has not been evaluated previously by the Committee. At its present meeting, the Committee considered the safety of sodium dichloroisocyanurate in relation to its possible use as a disinfectant for drinking-water in emergency situations, and for routine use in some water supplies.

When sodium dichloroisocyanurate is added to water, it is rapidly hydrolysed to release free available chlorine, establishing a complex series of equilibria involving six chlorinated and four non-chlorinated isocyanurates. As free available chlorine is consumed by reaction with organic material in the water, chloroisocyanurates will rapidly dissociate and continue to release free chlorine. Conventional chlorination of drinking-water with elemental chlorine gives rise to a number of by-products as a result of the reaction of free available chlorine with natural organic matter. The safety of these by-products has been addressed by WHO, with the development of guidelines for drinking-water quality. The use of sodium dichloroisocyanurate as a source of free available chlorine is not expected to lead to greater production of such by-products than does the use of elemental chlorine.

In contact with saliva of about pH 7.0, chlorinated isocyanurates react extremely rapidly such that, at the concentrations required to deliver free available chlorine at the levels typically used in drinking-water, no detectable chlorinated isocyanurate remains. The material that reaches the gastrointestinal tract is, therefore, the unchlorinated cyanuric acid (Oxychem, 1997, 2000). The relevant toxicological studies cited refer to this compound.

2. BIOLOGICAL DATA

2.1 Biochemical aspects: absorption, distribution and excretion

2.1.1 Sodium dichloroisocyanurate

No information was available.

2.1.2 Sodium cyanurate

Rats

In a study in which groups of five male rats and five female rats were given an intravenous bolus dose of ^{14}C -labelled sodium cyanurate of 5 mg/kgbw, the compound was rapidly distributed through the body, with an elimination half-time of 30–40 min. Other groups were given a dose of 5 mg/kgbw or 500 mg/kgbw, by gavage. At a dose of 5 mg/kgbw, the cyanurate was almost completely absorbed and the peak blood concentrations occurred 15–30 min after administration. The elimination half-times were 30–40 min after the intravenous dose and 40–60 min after the oral dose. Eighty-five per cent and 80% of the administered dose in males and females respectively was excreted in the urine as the parent compound. At the peak blood concentration, cyanurate was distributed between plasma and blood cells in equal proportions.

As part of the same study, another group received oral doses of sodium cyanurate of 5 mg/kgbw per day for 14 days and were given an equivalent dose of ^{14}C -labelled sodium cyanurate on day 15. This produced a similar excretory profile to that in animals given a bolus dose by oral or intravenous administration, with most of the radioactivity being excreted in the urine within 6 h and only very small amounts being detected after 24 h, faecal excretion representing about 5% of the administered dose.

The pattern of excretion following a single oral bolus dose of 500 mg/kgbw was different, with 70% and 55% of the administered dose in males and females respectively appearing in the faeces. The peak blood concentration occurred 60 min after dosing and the elimination half-time was 122–148 min, but with most of the cyanurate being excreted in urine within 24 h. All of the radioactivity could be accounted for as cyanurate except in animals receiving either multiple doses or a single dose of 500 mg/kgbw, when <0.5% of the radioactivity excreted was not cyanurate and was excreted before the 24-h time point. It was not possible to identify the substance responsible, but the authors suggested that its appearance might be a consequence of microbial action. No radioactivity was exhaled as $^{14}\text{CO}_2$.

After 7 days, the level of radioactivity in the tissues was undetectable or at the limits of detection (Barbee et al., 1983, Chadwick et al., 1983).

Dogs

In a study of similar design in dogs, groups of four males and four females were given radiolabelled cyanurate as a single intravenous dose of 5 mg/kgbw or a single oral dose of 5 or 500 mg/kgbw. Blood samples were collected at regular intervals from two males and two females and faeces and urine were collected from a further two males and two females. At the completion of the collection period, the animals from which faeces and urine had been collected were killed and 14 tissues and the remains of the carcass were analysed for residual radioactivity. An additional group of two males and two females was given unlabelled cyanurate orally at a dose of 5 mg/kgbw per day for 14 days and an equivalent dose of radiolabelled cyanurate on day 15.

Most of the radiolabelled cyanurate was excreted in the urine within 12 h, with minor amounts appearing after 24 h in all the animals receiving a dose of 5 mg/kg bw. In three of the animals receiving multiple doses, between 6% and 13% of the administered dose was excreted in the faeces, while in the other animals no more than 2% was excreted by this route. Animals receiving a dose of 500 mg/kgbw excreted between 27% and 86% of the administered dose in the faeces and the remainder in the urine. The elimination half-life was 1.5–2.0 h for both doses. However, there was some difficulty in determining the elimination kinetics, which the authors ascribed to continued absorption from the gastrointestinal tract. There was no evidence from blood kinetics for a slower elimination phase.

All of the excreted material was present as unchanged cyanurate. The residual level of radioactivity in all tissues was below the sensitivity of the method used. The total recovery in excreta ranged from 81% to 100%, with a mean of 93%; recovery in faeces was negligible for animals receiving the low dose (Barbee et al., 1984, Chadwick et al., 1982).

These data are supported by a study in rats receiving [¹⁴C]cyanuric acid by oral administration; more than 99% of the radioactivity in urine comprised the parent substance. These authors also showed that no systemic radioactivity could be detected after percutaneous exposure (Inokuchi et al., 1978).

Humans

Absorption and excretion of cyanuric acid has been studied in long-distance swimmers exposed by swimming in pools disinfected with chlorinated isocyanurates, and in two volunteers given an unspecified solution of cyanuric acid orally. More than 98% of the administered dose was recovered unchanged in urine after 24 h. The half-life of excretion was about 3 h (Allen et al., 1982).

2.2 Toxicological studies

In contact with saliva at about pH 7.0, chlorinated isocyanurates react extremely rapidly such that, at the concentrations required to deliver free available

Table 1. Acute oral toxicity of cyanuric acid and sodium dichloroisocyanurate

Test material	Species	LD ₅₀ (mg/kg bw)
Cyanuric acid	Rat	>5000 (two studies) 7700 (one study)
	Rabbit	>10000 (one study)
Sodium dichloroisocyanurate	Rabbit	>10000 (one study)
	Rat	>7500 (two studies)

From Tice, R. (1997) (all references cited in this publication)

LLD, lowest lethal dose

chlorine at the levels typically used in drinking-water, no detectable chlorinated isocyanurate remains. The material that reaches the gastrointestinal tract is, therefore, the unchlorinated cyanuric acid. The relevant toxicological studies cited refer to this compound.

2.2.1 Acute toxicity

(a) Sodium dichloroisocyanurate

The acute oral LD₅₀ for sodium dichloroisocyanurate (dihydrate) in rats was 1823 mg/kg bw (95% CI, 1479–2166 mg/kg bw), the acute oral LD₅₀ in males and females being 2094 and 1671 mg/kg bw respectively. The acute dermal LD₅₀ in rabbits was >5000 mg/kg bw (Gargus, 1984, 1985).

(b) Sodium cyanurate

The acute oral toxicity of sodium cyanurate and cyanuric acid in mice, rats and rabbits is reported to be between 1500 mg/kg bw and 10000 mg/kg bw. The available data are summarized in Table 1.

2.2.2 Short-term studies of toxicity

(a) Sodium dichloroisocyanurate

In early, limited studies in rats, sodium dichloroisocyanurate was administered in the drinking-water to groups of five male and five female rats, at a concentration of 0, 400, 1200, 4000 or 8000 mg/l (equivalent to 50, 150, 500 or 1000 mg/kg bw per day) for 59 days. Mortality, laboured breathing, reduced body weight and a reduction in water consumption were observed in the groups receiving concentrations of 4000 or 8000 mg/l. Water consumption was also reduced in the groups receiving sodium dichloroisocyanurate at a concentration of 400 or 1200 mg/l. No histopathology was carried out, but animals in the group receiving 8000 mg/l exhibited an increased incidence of gastrointestinal bleeding at necropsy, although the site of bleeding was not identified. The NOEL was reported as 50 mg/kg bw per day in males and 130 mg/kg bw per day in females (Hammond et al., 1986).

In a subsequent study in which rats were given sodium dichloroisocyanurate in the diet at a concentration of 0, 2000, 6000 or 12000 mg/kg of feed (equivalent to 0, 100, 300 or 600 mg/kg bw per day) for 13 weeks, body weights and food consumption were reduced in the groups receiving sodium dichloroisocyanurate at 6000 and 12000 mg/kg of feed. Relative liver and kidney weights were also increased in these groups. No other treatment-related changes, including histopathological changes, were reported. The NOEL was 2000 mg/kg feed (100 mg/kg bw per day) (Hammond et al., 1986).

(b) *Sodium cyanurate*

Groups of 25 male and 25 female B6C3F₁ mice received drinking-water containing sodium cyanurate at a concentration of up to 5375 mg/l (the limit of solubility at pH 7.0), equivalent to 0, 252, 522 or 1500 mg/kg bw per day, for 13 weeks. Control groups were given either sodium hippurate at a concentration of 7769 mg/l (sodium control), or tap water. Five animals of each sex from each group were killed after 6 weeks; all other animals were killed after 13 weeks. All animals were subjected to clinical pathological, gross pathological and histopathological examinations, and organ weights were measured. Increased water consumption was noted in the group receiving a dose of cyanurate of 1500 mg/kg bw per day. Absolute and relative ovarian weights showed a dose-related increase, which was significant in the groups given doses of 522 and 1500 mg/kg bw per day, but the same finding was observed in the sodium control group. The only compound-related change reported was the occurrence of bladder calculi in two males in the group given the highest dose, 1500 mg/kg bw per day. The NOEL was 1792 mg/l (equivalent to 522 mg/kg bw per day) (Serota et al., 1982).

Groups of Charles River rats were given drinking-water containing sodium cyanurate at a concentration of 896, 1792 or 5375 mg/l, equivalent to 72, 145 or 495 mg/kg bw per day, for 13 weeks. Forty rats of each sex were assigned to each group receiving doses of 145 and 495 mg/kg bw per day and to a control group receiving tap water. Twenty-four rats of each sex were assigned to the group receiving 72 mg/kg bw per day and to a sodium control group receiving sodium hippurate (1792 mg/l). Measurement of haematological and biochemical parameters, and urine analysis were undertaken before the commencement of exposure and then at intervals of 2 weeks. Four males and four females from both control groups and from the group receiving 495 mg/kg bw per day were killed at weeks 2, 4, 6, 8, and 10 and the remainder of the animals were killed at week 13. Organ weights were measured and examinations of gross pathology and histopathology, with particular attention being paid to the kidney and urinary tract, were carried out on all animals. A number of male rats in the group given a dose of 495 mg/kg bw per day (weeks 6 and 8, 1/4; week 10, 2/4; week 13, 4/20) and one male from the group given a dose of 145 mg/kg bw per day at week 13 had epithelial hyperplasia of the bladder. The NOEL was 896 mg/l (72 mg/kg bw per day) in males and 1792 mg/l (495 mg/kg bw per day) in females. No treatment-related effects were observed in the kidney or in any other tissue (Rajasekaran et al., 1981).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) *Sodium dichloroisocyanurate*

No long-term studies of toxicity and carcinogenicity are available for sodium dichloroisocyanurate.

(b) *Sodium cyanurate*

Groups of 80 male and 80 female Charles River CD1 rats were given drinking-water containing sodium cyanurate at a concentration of 400, 1200, 2400 or 5375 mg/l, equivalent to about 0, 26, 77, 154 or 371 mg/kgbw per day, for a period of 2 years. An additional 20 rats of each sex per group were treated for 63 weeks and maintained until 104 weeks to examine recovery from any recorded changes. There were two control groups, a tap water control group and a sodium control group given 7768 mg/l of sodium hippurate. Ten animals were killed at 6, 12 and 18 months. Measurement of haematological and clinical chemistry parameters, urine analysis, examinations of gross pathology, histopathology and measurement of organ weights were performed on all animals in the control group and in the group receiving a dose of 371 mg/kgbw per day. More restricted examinations of pathology were performed for the other groups, although tissues were retained for subsequent examination if necessary. There were a number of changes in organ weights that were not consistent. At 12 months, the absolute and relative thyroid and parathyroid weights in males given doses of sodium cyanurate of 154 and 371 mg/kgbw per day were significantly lower ($p < 0.05$) than those of the negative controls. There appeared to be no substance-related increase in tumour incidence.

Lesions of the urinary tract and heart were reported in males at the high dose. These were reported to occur mostly in the first 12 months of the study and were more frequent in animals that died or were killed because they were moribund. Nine of the 11 males with heart lesions receiving a dose of 371 mg/kgbw per day that died or were killed in the first year of the study also showed calculi in and distension of the bladder. Although urinary calculi were not found in all animals showing urinary tract lesions and cardiovascular lesions, it was postulated that a number of these calculi had been lost in fixation and an expert panel observed calculi fragments in a number of histological slides (Cohen et al., 1999). Since urolithiasis was more frequent in males than in females at the high dose and as the urethra is longer in males, the urinary tract lesions would be consistent with the presence of calculi. The authors of the study report considered that the urinary lesions, consisting of hyperplasia, bleeding and inflammation of the urinary bladder epithelium, inflamed ureters and renal tubular nephrosis were probably related to calculi, and that the acute myocarditis, necrosis and vascular mineralization were secondary to uraemia caused by the urinary tract lesions. There was an increased incidence of splenic haemosiderosis in the males receiving the high dose in the first year of the study. There was also a slight reduction in survival in the males given a high dose when compared to the negative control. Haematological and clinical chemistry parameters and urine analysis were reported to be unremarkable. There was a dose-related increase in water intake in the treated and

sodium control groups, which was attributed to increased sodium intake. Water consumption in the groups maintained until week 104 returned to normal after cessation of treatment. The NOEL was 2400 mg/l (equivalent to 154 mg/kg bw per day) (International Research and Development Corporation, 1985).

In a similar 2-year study in which groups of 100 male and 100 female B6C3F₁ mice were given drinking-water containing sodium cyanurate at a concentration of 0, 100 (80 animals of each sex), 400, 1200 or 5375 mg/l (equivalent to about 0, 30, 110, 340 or 1523 mg/kg bw per day). A group of 80 males and 80 females was given sodium hippurate as a sodium control. Measurement of haematological and clinical chemistry parameters and urine analysis was carried out for 10 males and 10 females from each group before the start of treatment and at weeks 26, 52, 78 and 104. Ten males and 10 females from each group were sacrificed after weeks 27, 53 and 79 and, together with the animals participating in the studies of clinical pathology, were subjected to necropsy. Tissues were collected and the urinary tract and gross lesions from the control group and from the group receiving the high dose were subjected to histopathological examination. All animals found in extremis throughout the study were sacrificed and also subjected to gross and histopathological examination. At the termination of the study, in week 105, all remaining animals were sacrificed, subjected to gross pathological examination and the tissues collected into fixative. All of the tissues from the control group and the group receiving the high dose were subjected to examination by microscopy.

Survival was similar in all groups. There were no significant differences in body weights for males. Although body weights were generally lower in females in groups receiving sodium cyanurate at doses of 110, 340 or 1523 mg/kg bw per day and in the sodium control group, compared to the water control group up to week 24, the only significant difference was for the group receiving the high dose, at weeks 13 and 26. There was a dose-related increase in water consumption above that in the control group receiving water only in both males and females, but water intake was highest in the the sodium control group. There were no treatment-related changes in haematological or clinical chemistry parameters, or urine analysis. Distended or enlarged abdomens were noted in males in the groups receiving the high and intermediate doses and in the sodium control group compared to the water control group, beginning at week 15. A similar effect was observed in females, but with a lower frequency. There were no treatment-related changes in the incidence of tumour or histopathological lesions at any dose (Serota et al., 1986).

2.2.4 Reproductive toxicity

(a) Sodium dichloroisocyanurate

In a study in which sodium dichloroisocyanurate was administered by gavage to pregnant mice of strain dd on days 6–15 of gestation at doses of 0, 25, 100 or 400 mg/kg bw per day, about 50% mortality was observed in animals receiving the high dose and reduced body weight was observed in the survivors. There were no signs of fetotoxicity, but delayed ossification associated with maternal toxicity was observed in the group receiving the high dose. This appeared to be an effect of the reaction of chlorine with the gastrointestinal tract (Tani et al., 1980).

(b) *Sodium cyanurate*

Groups of 25 pregnant Charles River COB and CD rats given sodium cyanurate at a dose of 0, 200, 1000 or 5000 mg/kgbw per day, by gavage, on days 6–15 of gestation, showed no signs of toxicity and no effects were reported in the offspring. Two sodium control groups given sodium hippurate at doses of sodium equivalent to 1000 and 5000 mg/kgbw per day were also included in the study. There were no deaths except in the group receiving a high dose of sodium, in which 11 animals died, although the cause of death was not determined. Fetotoxic effects were also seen in this group (Laughlin et al., 1982).

In a study of teratology in which Dutch belted rabbits were given sodium cyanurate by gavage at a dose of 0, 50, 200 or 500 mg/kgbw per day on days 6–18 of gestation, maternal toxicity reflected by dose-related body-weight loss, was observed at doses of 200 and 500 mg/kgbw per day. Small decreases in mean fetal weight and crown–rump length were observed at 500 mg/kgbw per day, but these were not statistically significant (Consultox Laboratories Ltd, 1974).

In study of teratology in which groups of 20 pregnant New Zealand White rabbits were given doses of sodium cyanurate of 0, 50, 200 or 500 mg/kgbw per day on days 6–18 of gestation, no maternal toxicity was observed. Animals in the groups receiving doses of 200 and 500 mg/kgbw per day showed reduced body-weight gain or slight body-weight loss on days 12–19 of gestation. An increased incidence of postimplantation loss was observed in the group given 500 mg/kgbw per day, although this was within the range of historical controls. There was an increased incidence of hydrocephaly in the group receiving 500 mg/kgbw per day (number of cases of hydrocephaly, 3, 0, 3 and 9 per group, respectively), but there was no apparent difference in the number of litters affected (1, 0, 2 and 2 litters affected per group, respectively). Hydrocephaly is not an uncommon finding in this strain of rabbits and although it normally occurs at a lower incidence, it is not considered to be a treatment-related effect (Rodwell, 1990).

Drinking-water containing sodium cyanurate at a concentration of 400, 1200 or 5375 mg/l (equivalent to about 26, 77, or 100 mg/kgbw per day) was given to three generations of Charles River CD rats. Control groups were given either tap water or a solution of sodium hippurate, 8056 mg/l, as a sodium control. For each generation, 12 males and 24 females were selected using randomized procedures. The F₀ generation was mated only after a minimum of 100 days of treatment and the F₁ and F₂ parents were only mated after at least 120 days of treatment. The F₀ and F₁ parents were mated twice to produce a and b litters and the F₂ parents were mated once to produce the F₃ offspring. As with other studies, an increase in water consumption was noted in females given the high dose; this was also noted in females of the F₁ and F₂ generations in the sodium control groups. There were no consistent effects reported for the offspring and no effects on reproduction were reported that could be associated with the administration of sodium cyanurate. There were treatment-related calculi observed in the bladder in males of the F₂ generation in the group receiving the high dose (100 mg/kgbw per day). These were associated with epithelial hyperplasia or chronic cystitis in three of the affected animals. The NOEL for reproductive effects was identified by the authors as 100 mg/kgbw per day (Aldridge et al., 1985).

2.2.5 Genotoxicity

Sodium cyanurate was not found to have mutagenic activity, either in the presence or absence of exogenous metabolic activation, in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, at a concentration of up to 10000 µg/plate, or in mouse lymphoma cells, at a concentration of up to 2000 µg/ml. Neither did it induce sister chromatid exchanges in Chinese hamster ovary cells in vitro at a concentration of up to 1500 µg/ml. No effects were observed in an assay for cytogenetic alterations in bone marrow of rats in vivo at a dose of 5000 mg/kg (Gridley & Ross, 1980; Kirby et al., 1981; Sharma, 1981; Stewart, 1981; Hammond et al., 1983).

2.2.6 Special studies: Immunotoxicity

Dichloroisocyanurate was not found to be a skin sensitizer in guinea-pigs (Mappes, 1984).

2.3 Observations in humans

Although sodium dichloroisocyanurate is widely used as a disinfectant for swimming pools, it appears that no specific studies on the effects of this substance in humans have been carried out, apart from an early study on absorption and excretion in long-distance swimmers. In this study, absorption and excretion of cyanuric acid was studied in long-distance swimmers exposed by swimming in pools disinfected with chlorinated isocyanurates, and in two volunteers given a solution of cyanuric acid to drink (see also section 2.1). No correlation was found between excretion of cyanuric acid and urinary concentrations of γ -glutamyl transpeptidase, measured as a potential marker of nephropathy (Allen et al., 1982).

3. DIETARY INTAKE

A typical concentration of free available chlorine used for the treatment of drinking-water is 1 mg/l and normally the objective would be to achieve a residual of available chlorine of between 0.2 and 0.5 mg/l. As anhydrous sodium dichloroisocyanurate contains about 63% free available chlorine, a solution of sodium dichloroisocyanurate of 1.6 mg/l (or of the dihydrate, 1.8 mg/l) is equivalent to a solution of free available chlorine of 1 mg/l. Drinking-water becomes increasingly unpalatable as concentrations of free chlorine increase above this level. However, to overcome initial chlorine demand, disinfection using sodium dichloroisocyanurate might require higher initial doses, but not greater than double these quantities (i.e. 3.2 mg/l), according to WHO estimates. For emergency disinfection of raw or pre-treated (settled, coagulated, and/or filtered) drinking-water supplies (lakes, rivers, wells, etc.), sodium dichloroisocyanurate would be introduced to achieve an initial concentration of available chlorine of 10 mg/l and to maintain a concentration of 1 mg/l.

The default intakes of drinking-water currently used by WHO are 2 l per day for adults, 1 l per day for a 10-kg child, and 0.75 l per day for a 5-kg bottle-fed

infant. WHO also recognizes that higher rates of intake may occur in some tropical countries. These intakes include water consumed in the form of juices and other beverages containing tap water (e.g. coffee), but no chlorinated isocyanurate would remain in these beverages. Thus, the daily intake of the dissociation products of sodium dichloroisocyanurate from the consumption of water by adults, children and infants, assuming a maximum application of sodium dichloroisocyanurate of 3.2 mg/l, would be equivalent to 6.4, 3.2, and 2.4 mg/person per day, expressed as sodium dichloroisocyanurate, respectively. Given that 1 mole of sodium dichloroisocyanurate corresponds to 1 mole of cyanuric acid (the ultimate product of the application of sodium dichloroisocyanurate), ingestion of cyanuric acid is estimated to be 0.06 mg/kg bw per day for adults, 0.19 mg/kg bw per day for children, and 0.28 mg/kg bw per day for a bottle-fed infant (WHO, 1993, WHO, 2000; Oxychem, 2003).

4. COMMENTS

In studies in which ^{14}C -labelled sodium cyanurate was administered in multiple doses of 5 mg/kg bw to rats, the sodium cyanurate was extensively absorbed and excreted unchanged in the urine, mainly within about 6 h. Only 5% of the administered dose was detected in the faeces and the radiolabel was not exhaled as $^{14}\text{CO}_2$. In a similar study in dogs, between 2% and 13% of ^{14}C -labelled sodium cyanurate was excreted unchanged in the faeces and the remainder in the urine, mainly within 12 h. In two human volunteers given a solution of cyanuric acid of unspecified concentration, >98% of the cyanurate was recovered unchanged in the urine after 24 h. The elimination half-life was 40–60 min in the rat, 1.5–2.0 h in the dog and about 3 h in humans.

Both sodium dichloroisocyanurate and sodium cyanurate have low acute oral toxicity.

In 13-week studies in mice given drinking-water containing sodium cyanurate at a concentration of up to 5375 mg/l (equivalent to 1500 mg/kg bw per day), the only compound-related effect reported was the occurrence of bladder calculi in males receiving the highest dose. In a similar study in Charles River rats, 1/28 males in the group receiving sodium cyanurate at a concentration of 1792 mg/l (equivalent to 145 mg/kg bw per day) and 7/28 males in the group receiving the highest dose (equivalent to 495 mg/kg bw per day) showed epithelial hyperplasia of the bladder.

In a 2-year study, Charles River CD1 rats were given drinking-water containing sodium cyanurate at a dose estimated as 26, 77, 154 or 371 mg/kg bw per day, with control groups receiving drinking-water containing an equivalent amount of sodium hippurate, or untreated drinking-water. Survival was slightly lower in the group receiving the highest dose compared to the control group receiving untreated drinking-water, but not the control group receiving sodium hippurate. There was no substance-related increase in tumour incidence. Multiple lesions of the urinary tract (calculi and hyperplasia, bleeding and inflammation of the bladder epithelium, dilated and inflamed ureters and renal tubular nephrosis) and cardiac lesions (acute myocarditis, necrosis and vascular mineralization) were reported in

males that died during the first year of the study and that were receiving a dose of 371 mg/kgbw per day. No toxicologically significant treatment-related effects were observed at 154 mg/kgbw per day, which was considered to be the NOEL in this study. In a similar 2-year study in which B6C3F₁ mice received a dose of sodium cyanurate equivalent to 30, 110, 340 or 1523 mg/kgbw per day, survival was similar in all groups and there were no treatment-related changes in the incidence of tumours or other histopathological lesions.

There were no signs of toxicity in adult animals and no effects reported in the offspring of groups of Charles River COB and CD rats given sodium cyanurate at doses of 0, 200, 1000 or 5000 mg/kgbw per day, by gavage, on days 6–15 of gestation. In studies in pregnant rabbits, either Dutch belted or New Zealand White, in which a dose of 0, 50, 200 or 500 mg/kgbw per day of sodium cyanurate were administered by gavage on days 6–18 of gestation, a small reduction in body-weight gain was observed in the groups receiving the two highest doses on days 12–19 of gestation in New Zealand White rabbits only, but compensatory body-weight gains were made by the end of the study. An increased incidence of post-implantation loss, which was within the historical control range, was also observed in this strain at 500 mg/kgbw. The Committee considered that these effects were not significant and there were no other effects that were considered to be related to treatment.

Three generations of Charles River CD rats were given drinking-water containing sodium cyanurate at an estimated dose of 26, 77 or 100 mg/kgbw per day, with control groups receiving untreated drinking-water or sodium hippurate. There were no treatment-related effects on reproductive parameters in the P₀, F₁ and F₂ generations or on offspring of the F₁, F₂ or F₃ generations.

Sodium cyanurate was not genotoxic in four different tests.

5. EVALUATION

The Committee concluded that studies of the toxicity of sodium cyanurate were appropriate for assessing the safety of sodium dichloroisocyanurate, because any residues of intact sodium dichloroisocyanurate in drinking-water would be rapidly converted to cyanuric acid on contact with saliva. Sodium cyanurate did not induce any genotoxic, carcinogenic or teratogenic effects.

The NOEL for sodium cyanurate derived from the 2-year study in rats was 154 mg/kgbw per day, equivalent to 220 mg/kgbw per day as anhydrous sodium dichloroisocyanurate. With the application of an uncertainty factor of 100, a tolerable daily intake of anhydrous sodium dichloroisocyanurate of 0–2.0 mg/kgbw per day for intake from drinking-water treated with sodium dichloroisocyanurate for the purpose of disinfection was determined by the Committee.

The no-adverse-effect levels in the reported studies are summarized in Table 2.

Table 2. NOAELs for sodium dichloroisocyanurate and sodium

Species	Study	Route of administration	Concentration (mg/l)	Dose (mg/kg bw)	Key effect	Reference
<i>Sodium dichloroisocyanurate</i>						
Rat	59-day	Drinking-water	400	50	Gastrointestinal tract bleeding	Hammond et al. (1986)
	13-week	Diet	400	100	NA	Hammond et al. (1986)
Mouse	Teratology	Gavage	—	100	Mortality/gastrointestinal tract	Tani et al. (1980)
<i>Sodium cyanurate</i>						
Rat	13-week	Drinking-water	896	72	Urinary tract	Rajasekaran et al. (1981)
	Three-generation	Drinking-water	1200	100	Reproduction	Aldridge et al. (1985)
	Teratology	Gavage	—	5000	Highest dose tested	Laughlin et al. (1982)
	2-year	Drinking-water	2400	154	Urinary tract/heart	International Research Development Corporation (1985)
Rabbit	Teratology	Gavage	—	500	Highest dose tested	Consultox Laboratories 1974
Mouse	Teratology	Gavage	—	500	Highest dose tested	Rodwell 1990
	13-week	Drinking-water	1792	522	Bladder calculi	Serota et al. 1982
	2-year	Drinking-water	5375	1523	NA	Serota et al. 1986

NA, not applicable

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