SCIENTIFIC OPINION

Flavouring Group Evaluation 42: Iron containing organic substances from chemical group 30[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids[2]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

The Scientific Panel on Materials in Contact with Food, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Scientific Panel was asked to evaluate two flavouring substances in the Flavouring Group Evaluation 42 (FGE.42), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These two flavouring substances belong to chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The two candidate substances (ferric ammonium citrate [FL-no: 16.089] and ferrous lactate [FL-no: 16.096]) are organic non-haem iron complexes. The Panel considered it inappropriate to evaluate these two substances using the Procedure for the evaluation of flavouring substances, because in their Opinion, the Procedure is not sufficiently underpinned for the evaluation of metal containing compounds. Instead, the Panel decided to evaluate the safety of these flavouring substances on the basis of data on iron toxicity in general and on toxicity data for several iron salts and complexes, including the candidate substances.

Candidate substance [FL-no: 16.096] possesses a chiral centre and may occur as optical isomers. This substance has been presented without specifying the stereoisomeric composition.

The substances have not been reported to occur naturally. However, the substances are complexes or salts which are composed of naturally occurring substances (iron with ammonium and citrate or iron and lactate).

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

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In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. For ferric ammonium citrate the Panel has received more detailed information of its use. For this substance a more specific exposure estimation has been carried out.

According to the default MSDI approach, the two candidate substances in this group have intakes in Europe of 550 and 73 microgram/capita/day (96 or 15 μg Fe/capita/day; [FL-no: 16.089 and 16.096], respectively). Exposure estimates based on the mTAMDI approach are 8100 and 6400 microgram/person/day (1418 or 1325 μg Fe/person/day; [FL-no: 16.089 and 16.096], respectively. For ferric ammonium citrate [FL-no: 16.089] data were available allowing estimation of the exposure for a specifically exposed European sub-population. The conservative exposure estimate for this sub-population amounts to 23750 μg/person/day (4160 μg Fe/person/day).

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the two candidate substances, would result in a total intake of approximately 623 μg/capita/day (111 μg Fe/capita/day).

No animal toxicity data have been submitted for ferric ammonium citrate [FL-no: 16.089]. Some animal toxicity data have been submitted for ferrous lactate [FL-no: 16.096], and some additional information can be found in reviews by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA) and in public literature. However, the data available indicate that the candidate substances in this FGE will disintegrate upon ingestion or absorption to give iron and citrate, lactate and ammonium. It may thus be anticipated that any adverse effects of these substances would be related to the separate components, rather than the parent substances. As citrate, lactate and ammonium are very common natural and high through-put endogenous substances, from these ligands no adverse effects are expected at the anticipated levels of exposure. The possibility of adverse effects elicited by iron has been further considered.

For the essential nutrient iron, under normal conditions, a daily absorption of 0.8-1 mg/person/day is sufficient for men, and approximately 2.5 mg/person/day for women of reproductive age. The recommended quantities of ingested iron have been estimated as approximately 10 mg/person/day and 15-20 mg/person/day for men and women, respectively, assuming 10 % absorption in men and 10-20 % in women of reproductive age.

Transient gastrointestinal distress may be observed at dose levels of 50-60 mg medicinal Fe/day when taken as non-haem iron supplements. The limited data indicated that supplemental intake of iron at levels of 30 mg/day or more can be associated with high iron stores, but that no point (i.e. iron overload) can be defined when these high iron stores would become associated with an increased risk on adverse effects (e.g. liver fibrosis). Based on theoretical calculations, there are some indications that in humans iron overload might be possible after exposure to 60 mg Fe/day for 5 years. In people suffering from hereditary haemochromatosis (approximately 0.5 % of the population) iron overload may occur already at normal dietary iron levels due to a defective feed-back regulation of iron absorption. The limited human data available indicate that chronic exposure to 10-30 Fe mg/person/day may be anticipated not to be associated with iron-induced toxic responses in the normal population. Although this estimate should not be considered equivalent to an Upper level or TDI, it could be used as a reference for the evaluation of the safety of the candidate substances in this FGE.

The available data do not raise concern with respect to genotoxicity.

No safety concern is anticipated for exposure at the levels of the respective MSDIs (i.e. 15 or 96 μg Fe/capita/day for [FL-no: 16.096] and [FL-no: 16.089], respectively). The same applies for the combined exposure based on MSDI (111 μg Fe/capita/day). The exposure estimates at the levels of the mTAMDIs for both substances (i.e. 1325 or 1418 μg Fe/capita/day for [FL-no: 16.096] and [FL-no: 16.089], respectively) cover about 7-18 % of the advised daily iron intake for adults, and the exposure estimate for [FL- no: 16.089] for the Scottish population would cover even more, up to 52 % of the advised daily iron intake. For people with normal iron homeostasis these high levels of exposure should not raise a safety concern, not even if these levels are additional to a background exposure that would suffice to cover the daily iron requirement. However, for people suffering from hereditary haemochromatosis (inadequate down-regulation of iron absorption) a safety concern is concluded. In order to improve the safety assessment for ferrous lactate [FL-

no: 16.096] based on the mTAMDI exposure estimates, more detailed information on use levels and the foods in which these substances are used are required. For ferric ammonium citrate [FL-no: 16.089], the conservative exposure estimate for the Scottish subpopulation corresponds to a consumption of the particular drink of about 1 L per adult per day. That may be a large amount, but in comparison with other non-alcoholic drinks it is not an extreme amount.
In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Incomplete specifications have been provided for the two flavouring substances. For [FL-no: 16.089] no assay minimum and identification test have been provided. For [FL-no: 16.096] no identification test is available and no information on stereochemical composition has been submitted. Thus, the final evaluation of the materials of commerce cannot be performed for both candidate substances, pending further information.

KEY WORDS

Flavourings, safety, ferric ammonium citrate, ferrous lactate, iron.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all others in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.
Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).
After the completion of the evaluation programme the positive list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

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ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 42

1.1. Description

The present Flavouring Group Evaluation deals with two iron containing organic substances from chemical group 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). Ferric ammonium citrate [FL-no: 16.089] contains 16.5 – 18.5 % Fe and ferrous lactate [FL-no: 16.096] contains approximately 20 % Fe. The two flavouring substances under consideration, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

There is no JECFA evaluation of structurally related flavouring substances in this FGE. However, the two flavouring substances ferric ammonium citrate [FL-no: 16.089] and ferrous lactate [FL-no: 16.096] (candidate substances) have been granted GRAS status by US-FDA (FDA, 1988; FDA, 1996). Ferric ammonium citrate has been evaluated by the JECFA as a food additive (JECFA, 1986a). Ferrous lactate has also been evaluated by the JECFA as a food additive in 1989 (JECFA, 1990a). Both substances were included in the provisional maximum TDI (pMTDI) for iron (800 μg Fe/kg body weight (bw)/day) derived by the JECFA at their twenty seventh meeting (JECFA, 1983b). Both candidate substances may be used as source for iron for the treatment of iron deficiency. In addition, ferric ammonium citrate is also used as a food additive to fortify bread (Elwood *et al.*, 1968; Parfitt, 1999). In 2006 the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) considered the possibilities to derive a tolerable upper intake level for iron. The NDA concluded that derivation of such a figure was not feasible for iron, because of a poor correlation between iron intake and biochemical indicators of iron status, between biochemical indicators and actual body stores or between body stores and adverse effects (EFSA, 2006j). Similar conclusions have been reached by the Food Standards Agency in UK and the Bundesinstitut für Risikobewertung in Germany (EVM, 2003; Domke *et al.*, 2004; BfR, 2009).

The candidate substances under consideration in the present evaluation are listed in Table 1 and 2a. No hydrolysis products are identified. The substances may disintegrate into free iron atoms and ligands. These will be separately discussed in Section 5. No supporting substances have been suggested by the Flavour Industry. The toxicity of various other iron compounds has been evaluated by the JECFA (JECFA, 1983b) and EFSA (EFSA, 2006j; EFSA, 2006k). Where appropriate this information will be included.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variation of their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (Chemical Abstract Service number (CAS number), FLAVIS number etc.).

The candidate substance ferrous lactate [FL-no: 16.096] has an asymmetrical carbon atom and thus can exist as optical isomers. Information on stereoisomerism has not been submitted.

1.3. Natural Occurrence in Food

According to TNO none of the two flavouring substances, ferric ammonium citrate [FL-no: 16.089] and ferrous lactate [FL-no: 16.096] has been reported to occur naturally in any food items (TNO, 2000).

2. Specifications

Purity criteria for the two substances (ferrous lactate [FL-no: 16.096] and ferric ammonium citrate [FL-no: 16.089]) have been provided by the Flavour Industry (Flavour Industry, 2004b; Flavour Industry, 2005d) (Table 1).
For ferrous lactate, the Flavour Industry has specified that this substance contains 20.7 % of iron. From the molecular mass it can be calculated that one mole of the substance would contain 2 moles of crystal water.
Two forms of ferric ammonium citrate exist (Merck Index, 2006). The brown hydrated form exists as garnet-red transparent scales or granules, or as a brownish-yellow powder, and contains ~ 9 % NH3, 16.5-18.5 % Fe, and ~ 65 % hydrated citric acid. The green hydrated form exists as green transparent, deliquescent scales, granules, or as a powder, and contains ~7.5 % NH3, 14.5-16 % Fe, and ~75 % hydrated citric acid. Following the specifications as presented in Table 1 it appears that the first (brown) form is used as a chemically defined flavouring substance. The amounts of ammonium and citric acid as given above for the brown form will be used for the estimation of exposure to the two ligands. For these calulations it is asssumed that the missing 8.5 % is crystal water.
Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the information is adequate for the two candidate substances, except that an ID test is missing for both substances and for ferrous lactate [FL-no: 16.096] the stereochemistry has not been specified (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximized Survey-Derived Daily Intake” (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).
However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.
The Panel also noted that in contrast to the generally low per capita intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.
Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).
One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.
One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).
For ferric ammonium citrate data were submitted that would allow for a more specific estimation of exposure. These data and the resulting exposure estimate have been presented in Section 3.3. For ferrous lactate such a calculation is not possible because no more details on use are available.

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The Maximised Survey-Derived Daily Intake (MSDI (SCF, 1999)) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[3] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation 42 (FGE.42) the annual production volumes of the two candidate substances for use as flavouring substances in Europe were reported to be 600 and 4500 kg/year for ferrous lactate and ferric ammonium citrate, respectively (Flavour Industry, 2004b; Flavour Industry, 2005d).

On the basis of the annual volume of production reported for the two candidate substances, MSDI values for each of these flavourings have been estimated (Table 2). The estimated MSDI of ferrous lactate [FL-no: 16.096] from use as a flavouring substance is 73 microgram/capita/day, that of ferric ammonium citrate [FL-no: 16.089] is 550 microgram/capita/day (Table 2). Using the MSDIs and the specifications (see Section 2 and Table 1) it can be calculated that the exposure to ferrous lactate would correspond to 48.2 μg lactate/capita/day and 15 μg Fe/capita/day. For ferric ammonium citrate, based on the specification data the exposure would correspond to 357 μg citrate/capita/day, 49.5 μg ammonium/capita/day and 96 μg Fe/capita/day (crystal water excluded).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the two candidate substances, information on food categories and normal and maximum use levels[4,5,6] were submitted by the Flavour Industry (Flavour Industry, 2004b; Flavour Industry, 2005d; EFFA, 2007a). The two candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

[3] EU figure 375 millions (Eurostat, 1998). This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

[4] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

[5] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

[6] The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 01.0	Dairy products, excluding products of category 2	None
Category 02.0	Fats and oils, and fat emulsions (type water-in-oil)	None
Category 03.0	Edible ices, including sherbet and sorbet	None
Category 04.1	Processed fruits	None
Category 04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 05.0	Confectionery	None
Category 06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	None
Category 07.0	Bakery wares	None
Category 08.0	Meat and meat products, including poultry and game	[FL-no: 16.096]
Category 09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	None
Category 10.0	Eggs and egg products	None
Category 11.0	Sweeteners, including honey	None
Category 12.0	Salts, spices, soups, sauces, salads, protein products etc.	[FL-no: 16.096]
Category 13.0	Foodstuffs intended for particular nutritional uses.	None
Category 14.1	Non-alcoholic ("soft") beverages, excluding dairy products	[FL-no: 16.089]
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	None
Category 15.0	Ready-to-eat savouries	None
Category 16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	None

According to the Flavour Industry the normal use levels for the two candidate substances are 25 and 42 mg/kg food and the maximum use levels are 25 and 280 mg/kg (EFFA, 2002i; Flavour Industry, 2004b; Flavour Industry, 2005d; EFFA, 2007a).
The mTAMDI values for the two candidate substances are 6400 and 8100 microgram/person/day for [FL-no: 16.096] and [FL-no: 16.089], respectively. Using these mTAMDIs and the specifications (see Section 2 and Table 1) it can be calculated that the exposure to ferrous lactate would corresponds to 4230 μg lactate/capita/day and 1325 μg Fe/person/day. For ferric ammonium citrate, based on the specification data the exposure would correspond to 5270 μg citrate/person/day, 730 μg ammonium/capita/day and 1418 μg Fe/person/day (crystal water excluded).
For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

3.3. Intake Estimate for Ferric Ammonium Citrate Based on Additionally Submitted Data

The information submitted by the Flavour Industry (Flavour Industry, 2004b) permits a more specific estimation of the exposure to ferric ammonium citrate [FL-no: 16.089]. This flavouring substance is used in non-alcoholic beverages only and the annual production volume of this beverage (190 million litres) multiplied by the used concentration (25 mg/l) would cover the total production volume of this flavouring substance (4500 kg/year). It was stated by Industry that the particular drink is predominantly consumed in Scotland (Flavour Industry, 2004b) and from the data submitted it can be calculated that approximately 80 % of the annual production of this beverage is destined for the Scottish market. Based on this, it can be calculated that the average daily exposure of the Scottish population (~ 5.2 million inhabitants in 2007 (General Register Office for Scotland, 2008) would amount to ca. 1900 μg/person/day, assuming equal consumption of the drink for the whole of the population. A conservative estimate (consumption by only 10 % of the Scottish population of the whole of the production) would result in an estimate of ~ 23750 μg ferric ammonium citrate/person/day (ca. 4160 μg Fe/person/day); an amount that would correspond to a

daily consumption of about 1 L of the particular beverage. The exposure estimates for the citrate and ammonium would correspond to 15440 μg/person/day and 2140 μg/person/day, respectively (crystal water excluded).

4. Absorption, Distribution, Metabolism and Elimination

The toxicokinetics of the two candidate substances in this FGE have not extensively been studied. However, the few data available demonstrate that iron from these substances may be absorbed to an extent which does not deviate from non-haem iron present in the food in other forms (see also ANNEX III). Whether the unabsorbed iron is in ionic state or still associated with the ligands is not relevant for systemic toxicity as non-absorbed iron cannot interfere with systemic body functions. The ligands present in these two candidate substances (lactate, ammonium and citrate) will be absorbed as well. These ligands are substances that are normal endogenous intermediates of carbohydrate and amino acid metabolism and are formed in the body in large quantities. In addition they are naturally present in food in large amounts. These ligands will be included completely in the anabolic and catabolic process within the body, ultimately resulting in the formation of carbon dioxide, water and urea (Voet & Voet, 2004).
Absorption of iron is regulated via feed-back mechanisms and the percentage of an oral dose that will be absorbed depends on the iron status of the body. In iron deficiency, a larger portion of the dose will be absorbed than in iron replete status. In general an absorption percentage of about 10 % of an oral dose is taken as a reasonable estimate for non-haem iron. Iron included in haem is known to be absorbed to a larger extent (up to 30 % of the dose). Hardly any iron is lost from the body, except for some limited loss via the skin and GI tract (ca. 1 mg per day), and in women via menstrual bleeding (< 1.6 mg/day). As a result of this, the iron status of a person depends largely upon intake of iron and the status-dependent efficiency of the absorption (see also Annex III). There is virtually no (status-dependently-)regulated mechanism for iron elimination.

5. Safety Evaluation of the Flavouring Substances ferrous lactate [FL-no: 16.096] and ferric ammonium citrate [FL-no: 16.089]

Normally, chemically defined substances are evaluated following the SCF / EFSA Procedure for the Safety Evaluation of the Flavouring Substances (see ANNEX I). However, the Panel decided that the two candidate flavouring substances in this FGE should not be evaluated according to this Procedure for the following reasons:

1. The decision tree is not very well equipped to make assumptions on the presumed safety or unsafety of (organo-)metal substances and therefore the classification system is not completely discriminative. Therefore the Panel did not allocate these two candidate substances to a Cramer class.
2. In the database from which the No Observed Adverse effect Levels (NOAELs) are taken to derive the thresholds of toxicological concern for the three Cramer classes (see (Munro *et al.*, 1996)), metal-containing substances (either organic or inorganic) were not included, except for some salts of alkali and alkaline earth metals.
3. There is an enormous variability in toxicity of metals, not only with respect to the metals themselves, but also with respect to their chemical speciation (e.g. inorganic *vs.* organic). The Procedure is too generic to cover this variability adequately.
4. Several metals (among which iron) have essential biological functions, an aspect which is not taken into account in the Procedure at all.

Based on these considerations, the Panel decided it appropriate to evaluate the safety of the two candidate substances in this group on the basis of substance-specific information. Separate Opinions on the Safety of

the various ligands and iron have been released by SCF, JECFA and EFSA in the past and these may serve to support this safety evaluation.

Ligands

Citrate [from FL-no: 16.089]

Citrate is a very common food constituent e.g. in citrus fruits and has been used as a food additive for a very long time. This substance has been allocated an acceptable daily intake (ADI) "not limited" by JECFA in 1973 (JECFA, 1974d). Based on the assumed exposure to ferric ammonium citrate from non alcoholic beverages and the specifications (see Section 2 and Table 1), it can be calculated that the exposure to citrate from this source will be 357 μg/capita/day (calculated from the MSDI), 5270 μg/person/day (calculated from the mTAMDI) or 15440 μg/person/day (based on the exposure estimate of 23750 μg ferric ammonium citrate/person/day, a conservative estimate derived for the Scottish population (see Section 3.2). The amounts of citrate calculated here are negligible compared to the endogenous production of citric acid in humans (ca. 2 kg/day; (OECD, 2001)). These amounts of citrate are not of safety concern.

Ammonium [from FL-no: 16.089]

Ammonium is a very common endogenous substance. It is mainly released during the catabolism of amino acids. Other sources are the intestinal bacterial conversion of nitrogen (e.g. from amino acids) into ammonia and the ammonia / ammonium ions in various foods (e.g. up to 16.4 g/kg tilsit cheese). Ammonia is converted to urea in the liver and eliminated via the urine in amounts of ca. 8 – 20 g/day, depending on protein consumption (figure for an adult male; corresponding to 4.5 – 12 g of ammonia per day). Also some free ammonia (ca. 1 g/day) is eliminated via this route (Wray, 1976). Other aspects of ammonia metabolism have been recently reviewed in a previous Opinion of the AFC Panel on ammonia and two ammonium salts (EFSA, 2009n). In this review also aspects of ammonium toxicity have been addressed. No NOAEL is available for ammonia. The JECFA has derived ADIs "not limited" or "not specified" for ammonium chloride and bicarbonate, respectively (JECFA, 1967b; JECFA, 1980a; JECFA, 1982b). From the specifications (see Section 2 and Table 1) it can be calculated that the MSDI or mTAMDI for [FL-no: 16.089] would correspond to 49.5 μg ammonium/capita/day or 730 μg ammonium/person/day, respectively. The conservative exposure estimate for ferric ammonium citrate (23750 μg/person/day), derived for the consumption of beverages containing this substance by specific European subpopulation, would correspond to an exposure to ammonium of 2140 μg/person/day. These amounts of ammonium are not of safety concern.

Lactate [from FL-no: 16.096]

Similar to citrate and ammonium, lactate is an endogenous substance (in carbohydrate and amino acid metabolism) and a natural component of very many foods, in particular fruits and fermented milk products. Under conditions of heavy energy demand (and thus high oxygen need) skeletal muscles convert glucose anaerobically into lactic acid, which is excreted from the muscle cells into the blood. In the liver this lactic acid is reduced to glucose. Ultimately any absorbed lactic acid will be oxidised to give carbon dioxide and water. It has been mentioned that at very young age the metabolic break-down of D-lactate is reduced if not impossible. In 1973 the JECFA derived an ADI "not limited" for lactate and several salts (JECFA, 1974d). In 1991, this view was also supported by the SCF ((SCF, 1991); ADI "not specified") and later iterated in the evaluation of lactate and sodium lactate for poultry carcass treatment (EFSA, 2006l).The amount of lactate that may be absorbed from [FL-no: 16.096] may be estimated to be 48.2 μg/capita/day based on the MSDI and 4230 μg/person/day based on the mTAMDI for ferrous lactate. These amounts of lactate are not of safety concern.

Iron [from both FL-no: 16.096 and 16.089]

Limited data on the toxicokinetics of the candidate substances has been submitted and these data focus entirely on the administered iron. The studies have been summarised in Annex III (mainly studies on absorption) and Section 4.

No toxicity data have been submitted for ferric ammonium citrate [FL-no: 16.089]. However, some toxicity data have been submitted for ferrous lactate [FL-no: 16.096] which have been summarised in Section 8.

Iron, being an essential nutrient, has recently been considered by EFSA (EFSA, 2006j) for derivation of a tolerable upper intake level. A review of its metabolism and toxicity has been published by Papanikolaou and Pantopoulos (Papanikolaou & Pantopoulos, 2005). From these sources the following information has been taken.

Any absorbed iron will ultimately be included in transferrin and ferritin as storage proteins or in proteins with defined biological functions (e.g. enzymes, haemo- and myoglobin). Free iron ions can mediate the formation of reactive oxygen species and other reactive species via redox cycling. These reactive molecules are considered to be the ultimate toxic agents responsible for iron toxicity, rather than the iron itself. In iron-replete status, sequestration of iron in proteins like ferritin or transferrin prevents this redox cycling and thus protects the body from iron toxicity. In iron overload status free iron may be found in the body and in such a situation iron toxicity may be observed (EFSA, 2006j; Papanikolaou & Pantopoulos, 2005).

Under normal conditions, a daily absorption of 0.8-1 mg/person/day is sufficient for men and approximately 2.5 mg/person/day for women of reproductive age. The recommended quantities of ingested iron have been estimated as approximately10 mg/person/day and 15-20 mg/person/day for men and women, respectively, assuming 10 % absorption in men and 10-20 % in women of reproductive age (EFSA, 2006j). The UK Food Standards Agency (Expert Group on Vitamins and Minerals; EVM), has indicated, for guidance purposes only, that a supplemental intake of approximately 17 mg Fe/day (equivalent to 0.28 mg/kg body weight (bw)/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of the people (EVM, 2003). The German Bundesinstitut für Risikobewertung (BfR) has released an Opinion on the safety of iron supplementation and fortification (BfR, 2009). BfR identified patients suffering from hereditary or secondary haemachromatosis as risk groups. BfR also indicated that normal men, postmenopausal women and elderly persons may also have an increased risk for iron overload symptoms as a result from use of iron supplements or foods fortified with iron. However, no quantitative data are compiled in this assessment, but BfR (2009) considered that total iron intake via food should be in the range of their Recommended Daily Intake (10-15 mg/day; (Domke *et al.*, 2004)).

From the data a tolerable upper intake level could not be derived (EFSA, 2006j). However, the Opinion gives some information on exposure levels at which adverse effects have been reported. Lethalilties have been reported after acute oral exposure to 60 mg medicinal Fe/kg bw. Oral doses of 10-20 mg medicinal Fe/kg bw are not associated with acute systemic toxicity. Transient gastrointestinal distress may be observed at dose levels of 50-60 mg Fe/person/day when taken as non-haem iron supplements. The limited data indicated that supplemental intake of iron at levels of 30 mg/person/day or more can be associated with high iron stores, but that no point can be defined when these high iron stores would become associated with an increased risk on adverse effects (e.g. liver fibrosis). Nonetheless, the Opinion cites a study by Borgh-Iohnson and Petersson Grawe (1995) in which it was predicted exposure to 60 mg Fe/person/day for 5 years could lead to a serum ferritin value close to that seen in iron overload. In people suffering from hereditary haemochromatosis[7] (approximately 0.5 % of the population) iron overload may occur already at normal dietary iron levels due to a defective feed-back regulation of iron absorption.

No indications of iron overload were obtained in a controlled study in humans who took diets fortified with sodium ferric EDTAte, providing 8 mg Fe/person/day for two years. This amount was sufficient however to improve the Fe status of the participants (Ballot *et al.*, 1989). However, the participants suffered initially from iron-deficiency anaemia, which might have been related to low dietary exposure to Fe. In another study

[7] Hereditary haemochromatosis (HHC) is an autosomal trait for which both recessive and dominant forms have been identified, not all pertaining to one and the same gene. The classical form (HFE) correlates strongly with homozygous presentation of a C282Y in the *HFE* gene on chromosome 6 (depending on the study, 52-100 % of HHC patients are homozygous for this mutation). Another less common mutation is H63D in the same gene which is found homozygous in 1.5 % of HHC patients. 35 more mutant alleles of the *HFE* gene have been identified. Estimated avaerage carrier frequencies of the C282Y and H63D alleles are highest in Northern Europe population (*ca.* 10 and 22%, respectively), but may deviate considerably in other populations (Hanson *et al.*, 2001). HHC has a prevalence of about 0.2-0.5% in white populations but less than 0.1 in black populations. Hemochromatosis can lead to cirrhosis and other liver diseases, hepatocellular carcinoma, diabetes, cardiomyopathy, arthritis, hypopituitary hypogonadism, fatigue, joint pain, skin bronzing or graying, abdominal pain, impotence, amenorrhea, and cardiac arrhythmias. The most common early symptom is weakness or fatigue (Cogswell *et al.*, 1998)

two subjects received 10 mg (an individual with normal iron store) or 25 mg (an individual with low iron store) of iron per day (as ferrous sulphate) through their diets for 500 days. At the end of the study iron stores were assessed. The iron store of the person with low iron store was clearly increased. The iron store of the normal individual was not significantly changed (Sayers *et al.*, 1994).
Exposures to iron from ferrous lactate [FL-no: 16.096] and ferric ammonium citrate [FL-no: 16.089] can be estimated to be 15 or 96 μg Fe/capita/day, respectively, based on the MSDIs and 1325 or 1418 μg Fe/person/day, respectively, based on the mTAMDI. The conservative estimate for ferric ammonium citrate calculated for the Scottish population is 4160 μg Fe/person/day. No safety concern is anticipated for exposure at the levels of the respective MSDIs. The exposure estimates at the levels of the mTAMDIs for both substances cover about 7-18 % of the advised daily iron intake for adults, and the exposure estimate for [FL-no: 16.089] for the Scottish population would cover even more, up to 52 % of the advised daily iron intake.
For people with normal iron homeostasis these high levels of exposure should not raise a safety concern, not even if these levels are additional to a background exposure that would suffice to cover the daily iron requirement. However, for people suffering from hereditary haemochromatosis a safety concern is concluded (see also (Papanikolaou & Pantopoulos, 2005)).

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for the two candidate substances based on the mTAMDI range from 6400 to 8100 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.

Table 6.1 Estimated intakes of the flavouring substances and estimated intakes of iron based on the MSDI approach, the mTAMDI approach and specific data for the Scottish population.

FL-no	EU Register name	MSDI (μg/*capita*/day)		mTAMDI (μg/person/day)		Scottish population (μg/person/day)	
		substance	Fe	substance	Fe	substance	Fe
16.089	Ferric ammonium citrate	550	96	8100	1418	23 750	4160
16.096	Ferrous lactate	73	15	6400	1325	-	-

7. Considerations of Combined Intakes from Use as Flavouring Substances

In general, because of structural similarities of substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.
The total estimated combined daily per capita intake of structurally related flavourings is estimated by summing the MSDI for individual substances.
Calculation of the combined iron intake from ferric ammonium citrate and ferrous lactate according to the MSDI approach would result in a combined exposure estimate of 111 μg Fe/capita/day.

8. Toxicity

8.1. Acute Toxicity

No data on acute toxicity of ferric ammonium citrate was submitted. For ferrous lactate an LD50 of 147 mg/kg bw (ca. 30 mg Fe/kg body weight (bw)) administered via gavage to female Fairfield Webster mice has been reported (Eickholt & White, 1965).

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Repeated dose toxicity data were only submitted for ferrous lactate [FL-no: 16.096] (see Table IV.2).

In a study designed to investigate the effect of iron lactate overload on bone homeostasis, four groups of 10 male Sprague-Dawley rats of 6 weeks of age received either 0 or 50000 mg ferrous lactate/kg feed (equivalent to 0 or 2500 mg/kg bw/day[8]; approximately 438 mg Fe/kg bw/day[9]) in a commercial diet for 2 or 4 weeks (Matsushima *et al.*, 2001). The study only aimed at the clarification of the pathology of iron-overload induced bone lesions and is of limited relevance for risk assessment. After both 2 and 4 weeks of high iron intake, changes in body weights and changes in white and red blood cell counts were observed; the latter not considered biologically relevant. Blood biochemistry showed exposure–related changes in markers for bone formation (increased) and urinalysis showed changes in markers for bone resorption. Histological analysis of bone structures showed bone loss in the exposure group. A No Observed Adverse effect Level (NOAEL) was not determined in this single dose level study.

Iron lactate was administered to groups of 10 male and 10 female rats (5 weeks of age) through the diets, containing 0, 0.625, 1.25, 2.5 or 5 % of the test substance for three months. Levels of exposure were equivalent to 0, 625, 1250, 2500 or 5000 mg/kg bw/day[10] (corresponding to approx. 110, 219, 437 and 875 mg Fe/kg bw/day). Animals were observed daily for clinical signs; body weights and food consumption were measured once weekly. At study termination, haematology, histopathology, immunohistochemistry and serum IgE levels were evaluated. Faeces, darker than in controls, was the only clinical sign observed in all treatment groups. Body weight gain was decreased for the 5.0 % group animals starting with the first week of administration of iron lactate and final mean body weights were 70 and 90 % of controls for males and females, respectively. However, food consumption of treated groups was comparable to the respective male or female control group. Significant macroscopic changes were not observed in any organs, except for a dose-related trend in darker colour of the liver and spleen at necropsy in treated croups as compared to controls. Main histological changes in particular observed in the two highest dose groups, were an accumulation of excess iron in hepatocytes, renal tubular cells, macrophages in the reticuloendothelial system of liver, spleen, bone marrow and lymph nodes. Microscopic histopathology findings of the alimentary tract for the treated animals consisted of eosinophilic infiltration, increased number of globule leukocytes, accumulation of macrophages engulfing the brown pigments in the lamina propria and changes in the epithelial cells of the gastric mucosa. The histopathological features of the lesions in the alimentary canal and peripheral eosinophilia of the treated animals were similar to those of eosinophilic gastritis and enteritis colitis, or similar to changes seen in humans and animals due to iron overload. The negative immunohistochemical reaction for CD8 suggested that globule leukocytes[11] in gastric and intestinal mucosa are different from cells with cytotoxic activity, which may reflect species differences in function among cells with the same morphology. Changes in red (decrease) and white blood cells (decrease) and platelets

[8] The authors stated body weights of the animals of about 150–180 g which is rather heavy for animals of that age. For that reason the estimation of exposure is done on the basis of a conversion factor of 0.05 mg/kg bw/day/mg/kg feed rather than 0.1 mg/kg bw/day/mg/kg feed, which normally would be more appropriate for such young animals.

[9] Exposure to iron calculated from an iron contents of 17.5 % in the lactate. This content was specified by Imai *et al* (2002). In this study, iron lactate from the same source was used. As stated in Table 1, FL-no: 16.096 contains 20.7 % of iron. The difference in iron content between the ferrous lactate used in these studies and the ferrous lactate used as a flavouring substance can be explained by assuming that in the animal studies a trihydrate rather than a dihydrate was used.

[10] Calculation of exposure based on conversion factor of 0.1 mg/kg bw/day/mg/kg feed because of young age of the animals and the length of the study.

[11] Mononuclear cell with large eosinophilic cytoplasmic granules found in the intestinal epithelium.

(increase) and reticulocytes (increase) were particularly abundant at the highest level of exposure. The number of hemosiderin-laden macrophages increased in the interstitial tissues of testes, epididymus and thyroid, without degenerative changes. A slight elevation of ALT, AST and BUN at the highest dose level indicated liver and kidney damage, but this was not histopathologically confirmed in these or in other tissues demonstrating infiltration of hemosiderin-laden macrophages. The authors concluded that tissue damage is restricted to parenchymal cells with intracellular iron deposition but that even for these cells the level of damage is not dependent on the amount of iron deposition. Since some microscopic findings were evident to some degree in all treated groups, a clear NOEL was not determined in this study (Narama *et al.*, 1999a; Narama *et al.*, 1999b).

Takegawa *et al.* (Takegawa *et al.*, 1995) exposed male and female F344 rats (5/sex/group) to 0 or 2 % iron lactate (equivalent to 1000 mg iron lactate /kg bw/day[12]; approx 175 mg Fe/kg bw/day[7,13]) via the diet for a period of 26 weeks. At the end of the study the animals were examined for haematology, serum chemistry, organ weights (9 tissues) and thiobarbituric acid reactants in the liver, kidney and serum. No intercurrent death was observed. Body weights were slightly depressed in the exposure groups. The males of the exposure group showed anaemia. Increased splenic weights were observed in the exposure group in both sexes and increase kidney weights were observed in the exposed females. Iron deposition was observed in livers, kidneys and spleens of treated males and females and in the intestines of treated females. In livers and kidneys of exposed males and females and in the serum of the females an increase in thiobarbituric acid reactants, indicative of oxidative stress, was observed (only abstract and tables / figures in English available).

Imai et al. (Imai *et al.*, 2002) fed groups of 50 male and female rats basal diets containing 0, 1 or 2 % of iron lactate for 104 weeks (equal to approx. 83 and 168 (m) or ~ 91 and 187 mg (f) Fe/kg bw/day[7,12]) to investigate the possibility of a carcinogenic potential. The control diet contained 170 mg Fe/kg feed, resulting in an additional intake of ca. 8 and 9 mg Fe/kg bw/day in the males and females, respectively. In total 40 tissues were examined. The study was broadly in compliance with internationally accepted guidelines. No effects on feed intake or survival were observed. A five or ten % reduction in body weight was observed at the end of the study in the low and high dose groups of both sexes, respectively. No iron lactate-induced tumours were observed, although the incidences of pancreatic acinar cell and endometrial gland hyperplasias were increased in males and females, respectively, in the 2 % group. A slight to moderate accumulation of brown pigmentation was observed in livers (Kuppfer cells, macrophages), kidneys (proximal tubular epithelium) and spleen of the high dosed males and in liver (Kuppfer cells, macrophages), kidneys (proximal tubular epithelium), spleen and endometrium in females, and in the lamina propria of the glandular stomach, small and large intestine. To determine if the endometrial epithelium hyperplasia could be ascribed to an endocrine disruptive activity, in a second experiment, in an estrogen responsive rat pituitary tumour cell line, MtT/Se and a human breast cancer cell line, MCF-7, the estrogenic potential of iron lactate with regard to receptor binding affinity and ERE-reporter gene activation was examined. Results in both cases were negative. From the study a NOAEL of 1 % iron lactate in the diet equal to 83 mg Fe/kg bw/day can be derived. Taking into account the Fe content in the control feed, the total iron intake at this NOAEL was 91 mg/kg bw/day.

According to EFSA (EFSA, 2006j) there is no consistent evidence for a causal relationship between iron exposure and gastrointestinal tract tumours, cardiovascular disease or type II diabetes mellitus in humans. However, Papanikolaou and Pantopoulos (Papanikolaou & Pantopoulos, 2005) have argued that there is a clear association between iron overload and an increased risk for carcinogenesis, mainly based on information obtained from patients with hereditary haemochromatosis. These patients have an increased risk for hepatocellular carcinoma and also other forms of cancer have been correlated to this condition such as oesophageal cancer, skin melanoma and acute myeloid leukemia. These authors provide argumentation that the potential of iron as a carcinogenic agent appears to be primarily related to its ability to promote oxidative

[12] Calculation of exposure based on conversion factor of 0.05 mg/kg bw/day/mg/kg feed because of the body weight of the animals at the start of the study (*ca*. 100 g) and the length of the study.

[13] This study was only available as an abstract. The Fe content of the lactate was not specified and the source was also not mentioned. As there is some overlap between the authors of this study and the authors of the other toxicity studies with Fe-lactate, the same kind of Fe-lactate has been assumed; see also footnote 7.

stress. Indeed in animals overloaded with iron increases in thiobarbituric acid reactive species has been observed, which may indicate increased levels of lipid peroxidation and oxidative stress. From this and from the absence of iron-induced genotoxicity, it is concluded that under conditions of normal body iron status no increased tumour risk is expected.

8.3. Developmental / Reproductive Toxicity Studies

No data on reproductive toxicity of either of the candidate substances was submitted.

The JECFA (JECFA, 1983b) reported an eight-generation study in rats, one with dietary administered (poorly available) iron oxide (25 mg Fe/animal/day) over eight generations. No toxic effects were observed. Teratogenicity studies were performed with ferrous sulphate and ferric sodium pyrophosphate in rats and mice administered via intubation. No maternal toxicity or teratogenicity was observed with the sulphate at dose levels up to 160 mg/kg bw/day in mice or 200 mg/kg bw/day in rats. With the pyrophosphate at dose levels up to 160 mg/kg bw/day no maternal toxicity or teratogenicity was observed either.

8.4. Genotoxicity Studies

Genotoxicity test with the candidate substances as well as several iron salts have been presented in table IV.4 and IV.5.

With ferrous lactate gene mutation studies in Salmonella and Sacharomyces cerevisiae are available. For ferric ammonium citrate a gene mutation study in Salmonella and a test for chromosomal aberrations in Chinese hamster lung cells have been performed. In these studies negative results were obtained (JECFA, 1983b; Ishidate *et al.*, 1984).

In addition, a number of ferrous and ferric salts have been tested for mutagenicity using Salmonella typhimurium and Saccharomyces cerevisiae, with and without activation. Plate tests as well as suspension tests were run with the Salmonella strains. Ferric pyrophosphate, ferric orthophosphate and sodium ferric pyrophosphate were inactive in all the systems used. Ferrous sulfate was active in the suspension tests with activation. The results indicate that the active agent is a frame shift mutagen which strongly reverts strain TA1537. Ferrous gluconate was mutagenic for indicator strain TA1538 in activation tests with primate liver preparations. It was inactive in the other tests (Litton Bionetics, 1974, 1975a, 1975b, 1976a, 1976b; all cited by (JECFA, 1983b) and (EFSA, 2006j); no further details available). No genotoxicity was observed without metabolic activation.

Also in another series of tests with Salmonella, no indications were obtained for any genotoxic activity with ferrous sulphate, ferrous chloride or ferrous orthophosphate at concentrations up to 10000 μg/plate with or without metabolic activation. A positive response was obtained with ferrous fumarate in TA98 with rat and hamster S9 (Seifried *et al.*, 2006). A genotoxic response was observed with ferrous sulphate in a mouse lymphoma assay at 750 μg/ml without metabolic activation and at 6 μg/ml in presence of metabolic activation when the data were analysed against the criteria in use at the time when the study was done. However, re-evaluation of the data against the current criteria resulted in an equivocal or inconclusive response, respectively (Seifried *et al.*, 2006). Also with ferric orthophosphate a positive response was observed in the mouse lymphoma assay at 2 μg/ml, which was later interpreted as equivocal. Ferrous fumarate gave in this test system a positive response in absence of metabolic activation at 920–980 μg/ml, but not in the presence of metabolic activation. Ferrous chloride was negative in this test system (Seifried *et al.*, 2006).

In another mouse lymphoma assay no genotoxic response was observed with ferric chloride (McGregor *et al.*, 1988a). Furthermore, a positive result has been reported with ferrous sulphate in an in vitro chromosomal aberration test in CHL cells, but this positive result was obtained with inclusion of gaps and polyploidy (Ishidate *et al.*, 1984). While inclusion of gaps in the scoring is currently considered inappropriate, polyploidy is an effect related to thresholded interactions. This positive result is therefore of no relevance for the assessment of the genotoxic potential of ferrous sulphate. No indications of genotoxicity were obtained with ferric citrate in similar pro- and eukaryotic test systems (Salmonella and CHL cells; (Ishidate *et al.*, 1984)).

An in vivo micronucleus test was found only with ferrous sulphate, and this study did not provide evidence for genotoxic potential of ferrous sulphate (Hayashi *et al.*, 1988).

The available studies with the candidate substances did not indicate that these would have a genotoxic potential. The studies described above with other ferrous or ferric substances do not indicate such a potential either. In all tests available, iron has been added in a free ionic state, which is hardly representative for the way iron is present in the body. Under the test conditions redox-cycling of free iron cannot be excluded and generation of reactive oxygen species may very well have affected the results in some of the genotoxicity studies. The Panel noted that, as discussed by Papanikolaou and Pantopoulos (2005) such redox cycling is a well-known phenomenon of free iron, which also contributes to iron-induced general toxicity.

9. Conclusions

The two candidate substances (ferric ammonium citrate [FL-no: 16.089] and ferrous lactate [FL-no: 16.096]) are organic non-haem iron complexes belonging to chemical group 30. The Panel considered it inappropriate to evaluate these two substances using the Procedure for the evaluation of flavouring substances, because neither the classification according to Cramer et al. (1978) nor the thresholds of concern (Munro *et al.*, 1996) seem to be sufficiently underpinned for the evaluation of metal containing compounds. Instead, the Panel decided to evaluate the safety of these flavouring substances on the basis of data on iron toxicity in general and on toxicity data for several iron salts and complexes, including the candidate substances.

Candidate substance [FL-no: 16.096] possesses a chiral centre and may occur as optical isomers. This substance has been presented without specifying the stereoisomeric composition.

The substances have not been reported to occur naturally. However, the substances are complexes or salts which are composed of naturally occurring substances (iron with ammonium and with citrate and iron with lactate).

According to the default MSDI approach, the two substances in this group have intakes in Europe of 550 and 73 microgram/capita/day (96 or 15 µg Fe/capita/day; [FL-no: 16.089 and 16.096], respectively). Exposure estimates based on the mTAMDI approach are 8100 and 6400 microgram/person/day (1418 and 1325 µg Fe/person/day; [FL-no: 16.089 and 16.096], respectively. For ferric ammonium citrate [FL-no: 16.089] data were available allowing estimation of the exposure for a specifically exposed European sub-population. The conservative exposure estimate for this sub-population amounts to 23750 µg/person/day (4160 µg Fe/person/day).

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the two candidate substances, would result in a total intake of approximately 623 µg/capita/day (111 µg Fe/capita/day).

No animal toxicity data have been submitted for ferric ammonium citrate [FL-no: 16.089]. Some animal toxicity data have been submitted for ferrous lactate [FL-no: 16.096], and some additional information can be found in reviews by the JECFA, EFSA and in public literature. However, the data available indicate that the candidate flavouring substances in this FGE will disintegrate upon ingestion or absorption to give iron and citrate, lactate and ammonium. It may thus be anticipated that any adverse effects of these substances would be related to the separate components, rather than the parent substances. As citrate, lactate and ammonium are very common natural and high through-put endogenous substances, from these ligands no adverse effects are expected at the anticipated levels of exposure. The possibility of adverse effects elicited by iron has been further considered.

For the essential nutrient iron, under normal conditions, a daily absorption of 0.8-1 mg/person/day is sufficient for men, and approximately 2.5 mg/person/day for women of reproductive age. The recommended quantities of ingested iron have been estimated as approximately 10 mg/person/day and 15-20 mg/person/day for men and women, respectively, assuming 10 % absorption in men and 10-20 % in women of reproductive age.

Transient gastrointestinal distress may be observed at dose levels of 50-60 mg medicinal Fe/day when taken as non-haem iron supplements. The limited data indicated that supplemental intake of iron at levels of 30 mg/day or more can be associated with high iron stores, but that no point (i.e. iron overload) can be defined when these high iron stores would become associated with an increased risk on adverse effects (e.g. liver fibrosis). Based on theoretical calculations, there are some indications that in humans iron overload might be possible after exposure to 60 mg Fe/day for 5 years. In people suffering from hereditary haemochromatosis

(approximately 0.5 % of the population) iron overload may occur already at normal dietary iron levels due to a defective feed-back regulation of iron absorption. The limited human data available indicate that chronic exposure to 10-30 mg Fe/person/day may be anticipated not to be associated with iron-induced toxic responses in the normal population. Although this estimate should not be considered equivalent to an Upper level or TDI, it could be used as a reference for the evaluation of the safety of the candidate substances in this FGE.

The available data do not raise concern with respect to genotoxicity.

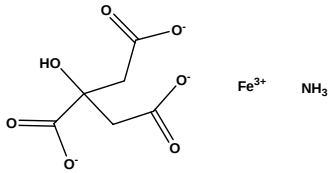
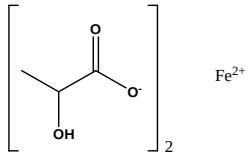
No safety concern is anticipated for exposure at the levels of the respective MSDIs (i.e. 15 or 96 μg Fe/capita/day for [FL-no: 16.096] and [FL-no: 16.089], respectively). The same applies for the combined exposure based on MSDI (111 μg Fe/capita/day). The exposure estimates at the levels of the mTAMDIs for both substances cover about 7-15 % of the advised daily iron intake for adults, and the exposure estimate for [FL-no: 16.089] for the Scottish population would cover even more, up to 52 % of the advised daily iron intake. For people with normal iron homeostasis these high levels of exposure should not raise a safety concern, not even if these levels are additional to a background exposure that would suffice to cover the daily iron requirement. However, for people suffering from hereditary haemochromatosis (inadequate down-regulation of iron absorption) a safety concern is concluded.

In order to improve the safety assessment for ferrous lactate [FL-no: 16.096] based on the mTAMDI exposure estimates, more detailed information on use levels and the foods in which these substances are used are required. For ferric ammonium citrate [FL-no: 16.089], the conservative exposure estimate for the Scottish subpopulation corresponds to a consumption of the particular drink of about 1 L per adult per day. That may be a large amount, but in comparison with other non-alcoholic drinks is not an extreme amount.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Incomplete specifications have been provided for the two candidate substances. For [FL-no: 16.089] no assay value and identification test have been provided. For [FL-no: 16.096] no identification test is available and no information on stereochemical composition has been submitted. Thus, the final evaluation of the materials of commerce cannot be performed for both candidate substances, pending further information.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 42

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 42

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
16.089	Ferric ammonium citrate		1185-57-5	Solid $C_6H_5O_7$ x Fe x NH_3	Very soluble Insoluble	Decomposes	n.a. n.a.	AV 7), ID 8). Contains 16.5-18.5 % Fe.
16.096	Ferrous lactate 6)		5905-52-2	Solid $(C_3H_5O_3)_2$ xFe, $2H_2O$ 270.02	Slightly soluble Practically insoluble or insoluble	n.a. 150-170 98 %	n.a. n.a.	ID 8). Contains 20.7 % Fe. Stereochemistry not specified. The indicated iron content corresponds to the dihydrate.

n.a.: not applicable.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95% ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

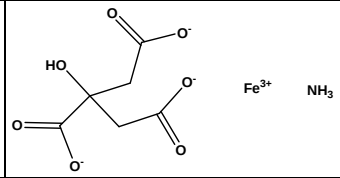
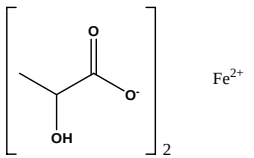
6) Stereoisomeric composition not specified.

7) AV: Missing minimum assay value.

8) ID: Missing identification test.

TABLE 2: SUMMARY OF SAFETY EVALUATION

Table 2: Summary of Safety Evaluation (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound	Outcome on the material of commerce [4), 5), or 6)]	Evaluation remarks
16.089	Ferric ammonium citrate		550	The Panel did not allocate a Cramer class. The substance is not evaluated using the Procedure.	A safety concern has been identified. 4)	6)	
16.096	Ferrous lactate		73	The Panel did not allocate a Cramer class. The substance is not evaluated using the Procedure.	A safety concern has been identified. 5)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) The safety concern was identified for people suffering from hereditary haemochromatosis when exposed at the level of mTAMDI and in particular for such persons belonging to a European subpopulation in which a high exposure to ferric ammonium citrate may be anticipated. No safety concern was identified for exposures at the level of MSDI or for healthy persons exposed at either MSDI or mTAMDI levels.

5) The safety concern was identified for people suffering from hereditary haemochromatosis when exposed at the level of mTAMDI. No safety concern was identified for exposures at the level of MSDI or for healthy persons exposed at either MSDI or mTAMDI levels.

7) Tentatively, the conclusion on the named substance may be considered also applicable on the material of commerce, pending further information on a test for identification.

6) No conclusion can be drawn due to lack of information on the purity of the material of commerce, on information on stereoisomerism and on a test for identification.

APPENDICES

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer *et al.,* 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[14] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[15] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[14] *"Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).*

[15] *"Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).*

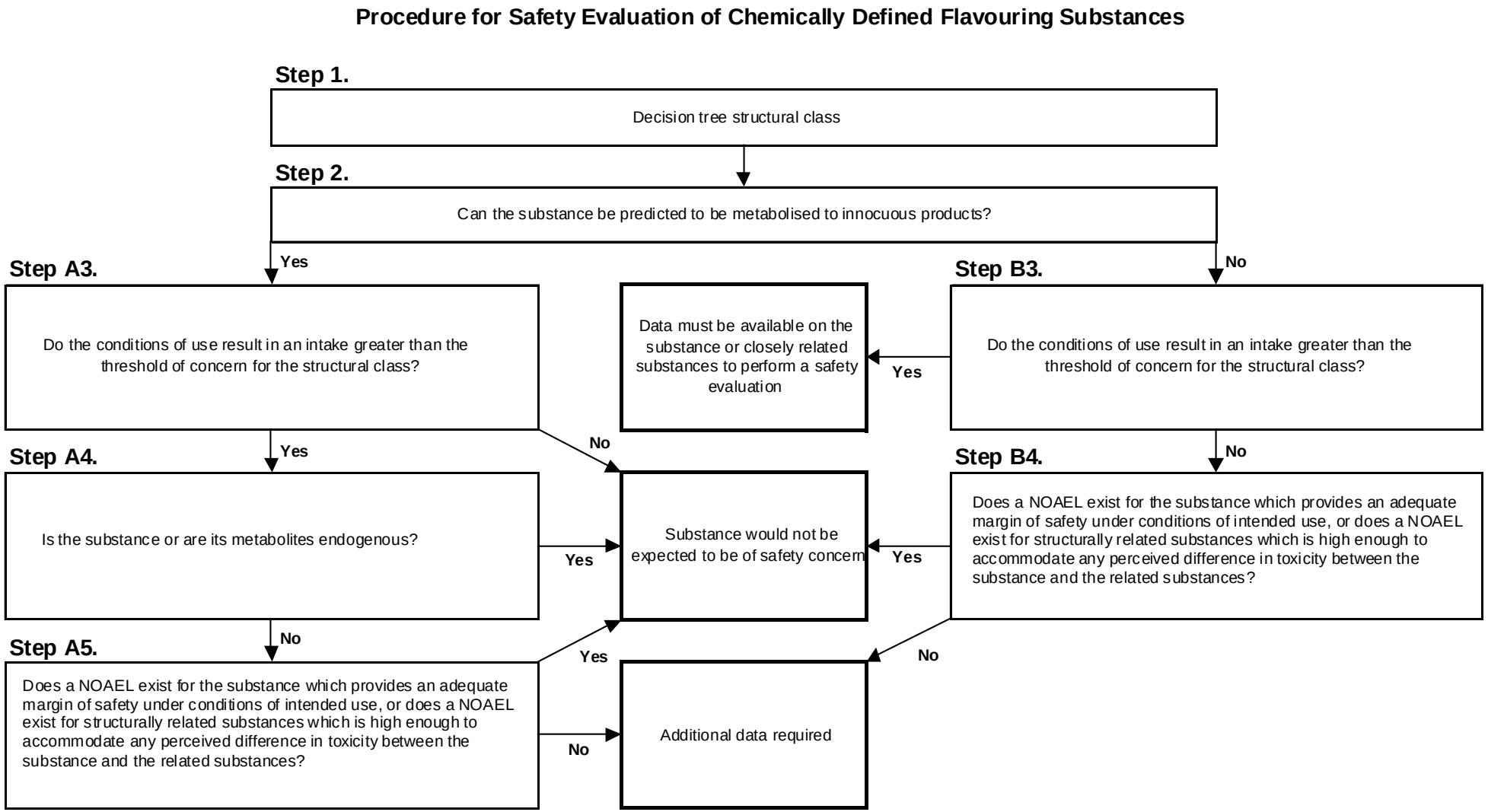


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1. Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories (EC, 2000a) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry for the two candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2.Normal and Maximum use levels (mg/kg) for candidate substances in FGE. 42 (Flavour Industry, 2004b; Flavour Industry, 2005d; EFFA, 2007a)

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
16.089	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	25 25	- -	- -	- -
16.096	- -	- -	- -	- -	- -	- -	- -	- -	42 280	- -	- -	- -	42 280	- -	- -	- -	- -	- -

II.2. mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person consumes the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No. 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for each of the two flavouring substances in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (Flavour Industry, 2004b; Flavour Industry, 2005d; EFFA, 2007a). The mTAMDI values are only given for highest reported normal use.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
16.089	Ferric ammonium citrate	8100		
16.096	Ferrous lactate	6400		

ANNEX III: METABOLISM

Introduction

No studies were submitted on the absorption of the two candidate substances in this FGE (ferrous lactate [FL-no: 16.096] and ferric ammonium citrate [FL-no: 16.089]). From the information submitted it is not clear if these two substances should be considered as (stable) complexes or as simple salts which may readily ionise in aqueous solution. Some data have been submitted on the absorption of iron from these substances (see below), and this may indicate that they are absorbed as such (i.e. as an intact complex) or as ions. Recently, the EFSA-AFC Panel has adopted an Opinion addressing the bioavailability of iron from a ferrous bisglycinate complex (EFSA, 2006k). From this Opinion it is clear that the iron of the bisglycinate complex will be released upon absorption. The molecular fragments released (i.e. Fe2+-ions and glycine) were subsequently included in the normal physiological pathways. If the two candidate substance behave similar to ferrous bisglycinate, they can be evaluated on the basis of the properties of their respective constituents, i.e. iron and lactic acid from [FL-no: 16.069] and iron, citrate and ammonium from [FL-no: 16.089].

Data on absorption of iron form the candidate substances

The bioaccessibility16 of free iron from various sources has been studied by Kapsokefalou et al. (Kapsokefalou *et al.,* 2005). Various iron sources, among which ferrous pyrophosphate, sulphate, gluconate, lactate and bisglycinate were added to milk samples which were subsequently subjected to enzymatic digestion. The bioaccessible free iron was determined in dialysis fluid. It appeared that total free iron (i.e. Fe2+ plus Fe3+) was equally accessible from all forms, but that the bisglycinate, the lactate and the pyrophosphate provided a better accessibility for the Fe2+ as compared to the sulphate and gluconate.

Iron absorption was studied by Walczyk et al. (Walczyk *et al.,* 2005), who determined erythrocyte incorporation of 57Fe and 58Fe from a rice and vegetable meal, seasoned with a 57Fe- or 58Fe-fortified fish sauce. The various iron isotopes were added as ferrous sulphate, ferrous lactate or ferric ammonium citrate. It appeared that iron absorption was 12-13 % from the sulphate as compared to 9 or 6 % from the lactate or ammonium citrate, respectively (geometric means). The authors referred also to studies (not submitted) in which absorption of iron from ferrous lactate showed similar or better absorption from the lactate as compared to sulphate. The discrepancy between study data and literature data was explained by assuming that in the fish sauce matrix conversion of Fe2+ into the less bioavailable Fe3+ might have occurred. In line with the results of the current study, various other studies (as referred to by the authors) showed reduced absorption of iron from ferric ammonium citrate as compared to ferrous sulphate.

Other information:

The bioavailability, biological functions (not further addressed here) and toxicity of iron have been extensively discussed by the EFSA (EFSA, 2006j; EFSA, 2006k) and (Papanikolaou & Pantopoulos, 2005). The information below is from these two references. No detailed referencing is included in these paragraphs. For further details the reader is referred to these overview documents.

Absorption

In the GI tract iron is generally considered to be present in two forms, haem-iron and non-haem ("inorganic") iron. Haem-iron may be absorbed as such, but immediately after absorption, it will be released from the haem structure. The absorption of haem iron is not very well understood and it is less well regulated by iron status than the absorption of non-haem-iron. Non-haem iron as Fe2+ is taken up by the intestinal mucosa by the divalent metal transporter (DMT1), the expression of which is higher in status of iron deficiency. Fe3+ is reduced to give Fe2+ before absorption. Absorption of haem-iron varies between 15 to 35 % in iron-replete or iron-deficient individuals, respectively. The absorption of non-haem iron seems to be highly variable, among others due to presence or absence of ligands. Weak ligands (e.g. lactate, ascorbic acid) may enhance inorganic iron absorption, while strong ligands (e.g. phytic acid) may reduce iron absorption. This suggests that ligand-bound non-haem iron may also be absorbed from the GI tract lumen

[16] Bioassessibility is the fraction of the ingested dose which is assessible for absorption. Bioavailability is the fraction of the dose that is absorbed, or more correctly the fraction of the dose that reaches the systemic circulation.

and be liberated inside the mucosal cells (EFSA, 2006k). In practice for non-haem iron the NDA Panel assumed 15 % absorption in young children, and 10 % absorption in adult males with normal iron status. For women of reproductive age 10-20 % absorption was assumed. However, the NDA Opinion also mentioned data showing up to 66 % absorption during pregnancy.

Transport / sequestration

Levels of free iron in the body are very low. Within the cells iron is bound to the sequestration protein ferritin, or to functional proteins such as enzymes (e.g. cytochromes) or haemoglobin. Alternatively intracellular iron can be bound to haemosiderin, which is a degradation product of ferritin. In the plasma iron ions are predominantly bound to transferrin, a protein with a high iron affinity. Low levels of ferritin can also be found in the plasma. Both serum ferritin and serum total iron binding capacity (reflecting transferrin) can be used as indicators of biomarkers for iron status.

Metabolism

Obviously, iron is not subject to biotransformation, but is also not an inert substance. Free iron ions or low molecular weight chelates can participate in redox cycling reactions and via Fenton- and Haber-Weiss reactions they can generate radical oxygen species (ROS). They can also facilitate formation of other radicals (thiyl; thiyl-peroxyl). Thus excess redox-active iron resulted in oxidative stress and in accelerated tissue degeneration, as can be observed in situations of prolonged iron overload (e.g. hereditary haemochromatosis or secondary haemochromatosis from repeated blood transfusions). Conversely, the transition for Fe^{2+} into Fe^{3+} and vice versa is used for the enzymatic transfer of electrons from one substance to another e.g. by cytochromes.

Homeostasis and Elimination

There is no specific physiological pathway for the body to eliminate (excessive) iron. Because of this, regulation of iron stores (2.2-3.8 g in an adult human body) depends fully on regulation of iron absorption. As a result of this, in individuals whose feedback on iron absorption is corrupted e.g. as a result of a genetic disorder, or as a result of repeated blood transfusion or chronic high-dose medical treatment with iron, iron overload may occur, which results in iron toxicity.

Some iron is lost via urine, but there is no control mechanism for that. Sloughing off of mucosal cells is another way to loose iron, but in fact this iron has not become systemically available. Some iron can also been lost via the skin. In total, via these pathways, approximately 1 mg of iron may be eliminated from the body of an adult male per day. For women, in addition to this 1 mg/day, iron loss via menstrual bleeding may amount up to 1.6 mg/day in 95 % of the female population.

Conclusion

The kinetics of the two candidate substances in this FGE has not extensively been studied. However, the few data available demonstrate that iron from these substances may be absorbed to an extent which does not deviate from non-haem iron present in the food in other forms. Whether the unabsorbed iron is in ionic state or still associated with the ligands is not that relevant as non-absorbed iron will not interfere with normal body functions. The absorbed iron will ultimately be included in transferrin and ferritin as storage proteins or in proteins with defined biological functions (e.g. enzymes, haemo- and myoglobin). In iron-replete status, sequestration of iron ions in proteins like ferritin or transferrin, protects the body from reactive oxygen species that could be formed from free iron ions as a result of redox cycling.

As stated above the ligands (lactate, ammonium and citrate) will be absorbed as well. These molecules are endogenous substances which will be completely included in the anabolic and catabolic processes within the body, ultimately resulting in the formation of carbon dioxide, water and urea.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 30

TABLE IV.1: ACUTE TOXICITY

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
Ferrous lactate [FL no: 16.096].	mice	Female	gavage	147 mg/kg bw (ca. 30 mg Fe/kg bw)	(Eickholt & White, 1965)	

Subacute / subchronic / chronic / carcinogenic toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 30.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
Ferrous lactate [FL no 16.096]	Sprague-Dawley; 10 m per group	Dietary	0 or 50000 mg/kg feed	2 or 4 weeks	None	(Matsushima *et al.*, 2001)	Limited study to investigate effect of iron overload on bone homeostasis.
	F344 rats 10 m/f per group	Dietary	0, 0.625, 1.25, 2.5 and 5 % (equivalent to 0, 110, 219, 437 and 875 mg Fe/kg bw/day)	13 weeks	None identified	(Narama *et al.*, 1999a; Narama *et al.*, 1999b)	Study aimed at clarification of iron overload effects.
	F344 rats 5 m/f per group	Dietary	0 and 2%	26 weeks	None identified	(Takegawa *et al.*, 1995)	Limited study with abstract and tables in English.
	F344/DuCrj rat, 50 f/m per group	Dietary	0, 1 and 2 % (equal to 0, 475 and 963 (m) or 0, 524 and 1070 (f) mg/kg bw/day)[1]	104 weeks	1 %; (equivalent to 83 mg Fe/kg bw/day)	(Imai *et al.*, 2002)	Valid carcinogenicity study.

[1] Iron contents of diets: 17, 167 and 356 mg/100 g; equal to ~ 8, (83 +8) and (168+8) (m) or ~9, (91+9) and (187+9) mg (f) Fe/kg bw/day. Note that the Fe exposure at the NOAEL mentioned inn the table is for the lactate iron only.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental and reproductive toxicity data are available for the two candidate substances of the present flavouring group evaluation from chemical group 30.

In vitro mutagenicity/genotoxicity data are available for the two candidate substances of the present flavouring group evaluation from chemical group 30.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Ferrous lactate [16.096]	*Salmonella typhimurium*	Strains TA1535, TA1537, and TA1538	Not specified	Negative[1,2]	(JECFA, 1983b)	Both plate and suspension test were run. No further details available.
	Saccharomyces cerevisiae	Strain D-4	Not specified	Negative[1,2]	(JECFA, 1983b)	No further details available.
Ferric ammonium citrate [16.089]	*Salmonella typhimurium*	Strains TA 92, TA 94, TA98, TA100, TA1535 and TA1537	6 concentrations up to 100 mg/plate	Negative[1,2]	(Ishidate *et al.*, 1984)	Limitedly reported, but otherwise valid.
	Chinese Hamster lung cells	Chromosomal aberrations	0.25 mg/ml	Negative[2]	(Ishidate *et al.*, 1984)	Cells were exposed for 24 and 48 hrs. The highest dose tested caused 50 % cell death. Limitedly reported but otherwise valid.
(Ferric citrate)	*Salmonella typhimurium*	Strains TA 92, TA 94, TA98, TA100, TA1535 and TA1537	6 concentrations up to 25 mg/plate	Negative[1,2]	(Ishidate *et al.*, 1984)	Limitedly reported, but otherwise valid.
	Chinese Hamster lung cells	Chromosomal aberrations in CHL cells	0. 5 mg/ml	Negative[2]	(Ishidate *et al.*, 1984)	Cells were exposed for 24 and 48 hrs. The highest dose tested caused 50 % cell death. Limitedly reported but otherwise valid.
(Ferric pyrophosphate)	*Salmonella typhimurium*	Strains TA1535, TA1537, and TA1538	Not specified	Negative[1,2]	(JECFA, 1983b)	Both plate and suspension test were run. No further details available.
	Saccharomyces cerevisiae	Strain D-4	Not specified	Negative[1,2]	(JECFA, 1983b)	No further details available.
(Ferric orthophosphate)	*Salmonella typhimurium*	Strains TA1535, TA1537, and TA1538	Not specified	Negative[1,2]	(JECFA, 1983b)	Both plate and suspension test were run. No further details available.
	Salmonella typhimurium	Strains TA98, TA100, TA102, TA1535, TA1537, TA1538	0 – 10000 µg/plate	Negative[1,2]	(Seifried *et al.*, 2006)	Valid.
	Saccharomyces cerevisiae	Strain D-4	Not specified	Negative[1,2]	(JECFA, 1983b)	No further details available.
	Mouse lymphoma cells	L5178Y ($Tk^{+/-}$)	0-1000 µg/ml[3] 0 – 5 µg/ml[3]	Negative[2] Positive[1]	(Seifried *et al.*, 2006)	Valid. Valid; lowest effective concentration 2 µg/ml. The indicated result is the original interpretation. According to the authors, against the current criteria the test was equivocal.
(Sodium ferric pyrophosphate)	*Salmonella typhimurium*	Strains TA1535, TA1537, and TA1538	Not specified	Negative[1,2]	(JECFA, 1983b)	Both plate and suspension test were run. No further details available.
	Saccharomyces cerevisiae	Strain D-4	Not specified	Negative[1,2]	(JECFA, 1983b)	No further details available.
(Ferrous sulphate)	*Salmonella typhimurium*	Strains TA1535, TA1537, and TA1538	Not specified	Positive	(JECFA, 1983b)	Both plate and suspension test were run. Positive in TA1537 with metabolic activation. No further details available.
	Salmonella typhimurium	Strains TA98, TA 100, TA1535, TA1537, and TA1538	0-10000 µg/plate	Negative[1,2]	(Seifried *et al.*, 2006)	Valid.
	Salmonella typhimurium	Strains TA 92, TA 94, TA98, TA100, TA1535 and TA1537	6 concentrations up to 10 mg/plate	Negative[1,2]	(Ishidate *et al.*, 1984)	Limitedly reported, but otherwise valid.
	Saccharomyces cerevisiae	Strain D-4	Not specified	Negative[1,2]	(JECFA, 1983b)	No further details available.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mouse lymphoma cells	L5178Y ($Tk^{+/-}$)	0-1000 μg/ml[3] 0 – 7.5 μg/ml[3]	Positive[2] Positive[1]	(Seifried *et al.*, 2006)	Valid; lowest effective concentration 750 μg/ml. The indicated result is the original interpretation. According to the authors, against current criteria the test was equivocal Valid; lowest effective concentration 6 μg/ml. The indicated result is the original interpretation. According to the authors, against the current criteria the test was inconclusive.
	Chinese Hamster lung cells	Chromosomal aberations	1.25 mg/ml	Positive[2]	(Ishidate *et al.*, 1984)	Cells were exposed for 24 and 48 hrs. The highest dose tested caused 50 % cell death. The positive results included polyploidy and gaps.
(Ferrous gluconate)	*Salmonella typhimurium*	Strains TA1535, TA1537, and TA1538	Not specified	positive	(JECFA, 1983b)	Both plate and suspension test were run. Positive in TA-1538 with metabolic activation. No further details available.
	Saccharomyces cerevisiae	Strain D-4	Not specified	Negative[1,2]	(JECFA, 1983b)	No further details available.
(Ferrous fumarate)	*Salmonella typhimurium*	Strains TA98, TA100, TA102	0 – 10000 μg/plate	Positive[2] Negative[1,2]	(Seifried *et al.*, 2006)	Valid. The positive result was obtained with TA98. In the other strains no indication of genotoxicity was obtained.
	Mouse lymphoma cells	L5178Y ($Tk^{+/-}$)	0 – 980 μg/ml[3] 0 – 14 μg/ml[3]	Positive[2] Negative[1]	(Seifried *et al.*, 2006)	Valid. The response was obtained with a concentration of 920 – 980 μg/ml. Valid. The indicated result is the original interpretation. According to the authors, against the current criteria the test was inconclusive.
(Ferric chloride)	*Salmonella typhimurium*	Strains TA98, TA100, TA102, TA1535, TA1537, TA1538	0 – 10000 μg/plate	Negative[1,2]	(Seifried *et al.*, 2006)	Valid.
	Mouse lymphoma cells	L5178Y ($Tk^{+/-}$)	0 -1200 μg/ml 0 – 140 μg/ml	Negative[2] Negative[1]	(McGregor *et al.*, 1988a)	Each test was run twice. Precipitaion was observed at 150 μg/ml and above. A < two fold increase was observed at 600 μg/ml. This concentration was only tested once. 1200 μg/ml was lethal to the cells.
	Mouse lymphoma cells	L5178Y ($Tk^{+/-}$)	0 – 5000 μg/ml[3] 0 – 6 μg/ml[3]	Negative[2] Negative[1]	(Seifried *et al.*, 2006)	Valid. Valid. The indicated result is the original interpretation. According to the authors, against the current criteria the test was inconclusive.

1 with metabolic activation.

2 without metabolic activation.

3 the maximal concentrations tested were limted by strong reduction of clonal growth (~ 90% reduction).

In vivo mutagenicity/genotoxicity data are available for none of the candidate substance of the present flavouring group evaluation from chemical group 30.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Ferrous sulphate)	Mouse; ddY strain; 6m/treatment	Micronuclei in bone marrow erythrocytes	i.p.	0, 100, 150 mg/kg bw 0, 25, 50, 100, 180 mg/kg bw 4 times 50 mg/kg bw	Negative Negative Negative	(Hayashi *et al*., 1988)	1000 PCEs were scored. PCE/NCE ratio not affected; cells harvested 24 h after last treatment. In one run, at 100 mg/kg bw a two fold increase in MNPCEs was found. No increase was seen at 180 mg/kg bw (4/6 animals died).

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ABBREVIATIONS

ADI	Acceptable Daily Intake
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
No	Number
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NTP	National Toxicology Program
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
WHO	World Health Organisation