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Re-evaluation of sodium ferrocyanide (E 535), potassium ferrocyanide (E 536) and calcium ferrocyanide (E 538) as food additives

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Abstract

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provided a scientific opinion re-evaluating the safety of sodium ferrocyanide (E 535), potassium ferrocyanide (E 536), and evaluating the safety of calcium ferrocyanide (E 538) as food additives. The Panel considered that adequate exposure and toxicity data were available. Ferrocyanides (E 535–538) are solely authorised in two food categories as salt substitutes. To assess the dietary exposure to ferrocyanides (E 535–538) from their use as food additives, the exposure was calculated based on regulatory maximum level exposure assessment scenario (maximum permitted level (MPL)) and the refined exposure assessment scenario. Dietary exposure to ferrocyanides was calculated based on mean and high levels consumption of salts in both the regulatory maximum level and the refined scenario. In the MPL scenario, the exposure to ferrocyanides (E 535–538) from their use as a food additive was up to 0.009 mg/kg body weight (bw) per day in children and adolescents. In the refined estimated exposure scenario, the exposure was up to 0.003 mg/kg bw per day in children and adolescents. Absorption of ferrocyanides is low and there is no accumulation in human. There is no concern with respect to genotoxicity and carcinogenicity. Reproductive studies were not available, but a no observed adverse effect level (NOAEL) of 1,000 mg sodium ferrocyanide/kg bw per day (highest dose tested) was identified from a prenatal developmental toxicity study. The kidney appeared to be the target organ for ferrocyanides toxicity and 4.4 mg sodium ferrocyanide/kg bw per day was identified as the NOAEL for the renal effects in a chronic (2-year) study in rats. Assuming that the toxicity of this compound is due to the ferrocyanide ion only, the Panel established a group acceptable daily intake (ADI) for sodium, potassium and calcium ferrocyanide of 0.03 mg/kg bw per day expressed as ferrocyanide ion. The Panel concluded that ferrocyanides (E 535–538) are of no safety concern at the current authorised use and use levels.

Keywords: ferrocyanide, E 535, E 536, E 538, sodium, potassium, calcium

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Note to the amendment: The correction made regards the addition of "00" before each of the three question numbers (on page 1). *To avoid confusion, the original version of the opinion has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made*.

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Summary

The present opinion deals with the re-evaluation of the safety of sodium ferrocyanide (E 535), potassium ferrocyanide (E 536), and evaluation of the safety of calcium ferrocyanide (E 538) as food additives.

Sodium, potassium and calcium ferrocyanides (E 535, E 536 and E 538) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012[1].

In the EU, sodium and potassium ferrocyanide, used as food additives, were previously evaluated by the Scientific Committee on Food (SCF) in 1990. In that evaluation, the SCF agreed with the acceptable daily intake (ADI) of 0.025 mg/kg body weight (bw) per day (calculated as sodium ferrocyanide) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for sodium and potassium ferrocyanide.

Sodium, potassium and calcium ferrocyanide were evaluated by JECFA in 1969, 1973 and 1974. A temporary acceptance of 0–0.00125 mg/kg bw per day was established in 1969 based on a dietary level of 0.05% sodium ferrocyanide and subsequently a temporary ADI of 0–0.025 mg/kg bw per day was established. In 1974, the temporary ADI of 0–0.025 mg/kg per bw (calculated as sodium ferrocyanide) was confirmed. A larger uncertainty factor (1,000) than the generally one employed was used to compensate for the absence of a long-term feeding study.

Potassium and sodium ferrocyanide were evaluated by the UK Committees on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in 1994 and set a group ADI for ferrocyanides of 0–0.05 mg/kg bw per day.

The Scientific Committee for Animal Nutrition (SCAN) evaluated the safety for the target animals, the users, the workers, the consumers and the environment of sodium and potassium ferrocyanide used as anticaking agents. It was concluded that sodium and potassium ferrocyanide in salt for feed use (20, 80 and 100 mg/kg in salt for man, poultry and livestock, respectively) is acceptable in regard to the safety for target animals and human consumers.

Sodium, potassium and calcium ferrocyanide were evaluated by a working group established by the Nordic Council of Ministers in 2000. Sodium, potassium and calcium ferrocyanide were not considered to cause a safety problem due to the very small quantities consumed.

Potassium ferrocyanide is absorbed to a limited extent from the gastrointestinal tract following oral administration to rats and in humans absorption is low (0.25–0.42%). Potassium ferrocyanide is of low acute oral toxicity. Based on the available data, the Panel considered that the use of ferrocyanides as food additives is not of genotoxic concern and that ferrocyanides are not carcinogenic. Reproductive studies were not available, but a no observed adverse effect level (NOAEL) of 1,000 mg sodium ferrocyanide/kg bw per day (highest dose tested) was identified from a prenatal developmental toxicity study.

In a 2-year study, animals frequently showed a higher cell excretion rate in urine samples compared to controls. Since the kidney is known to be the target organ for ferrocyanide toxicity, the Panel considered the increased cell excretion rate indicative for occasional, transient kidney toxicity and identified a NOAEL of 4.4 mg/kg bw per day. Based on this NOAEL of 4.4 mg sodium ferrocyanide/kg bw per day for male rats, the Panel derived an ADI of 0.044 mg sodium ferrocyanide/kg bw per day. Assuming that the toxicity of this compound is due to the ferrocyanide ion only, the Panel established a group ADI for sodium, potassium and calcium ferrocyanide of 0.03 mg/kg bw per day expressed as ferrocyanide ion. The Panel noted that at this ADI the potential amount of free cyanide released would not be of safety concern.

To assess the dietary exposure to ferrocyanides (E 535–538) from their use as food additives, the exposure was calculated based on (1) maximum permitted level (MPL) in FC 12.1.1 'Salt' set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the mean reported use levels of salt (defined as the *refined exposure assessment scenario*).

The Panel decided to use salt intake data from urinary excretion studies for the assessment of exposure to ferrocyanides (E 535–538) instead of the food consumption data from the EFSA Comprehensive European Food Consumption Database as dietary surveys are commonly not considered as a good source of information in the estimation of salt intake while a more accurate way of estimation of the salt intake is a calculation from the urinary excretion of sodium.

[1] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1.

Dietary exposure to ferrocyanides was calculated based on mean and high levels consumption of salts in both the regulatory maximum level and the refined scenario.

In the *MPL scenario*, the exposure to ferrocyanides (E 535–538) from their use as a food additive was up to 0.009 mg/kg bw per day in children and adolescents. In the *refined estimated exposure scenario,* the exposure was up to 0.004 mg/kg bw per day in children and adolescents. Considering that the majority of the use levels in salt reported by Industry were for sodium ferrocyanide (E 535), these exposures would correspond approximately to 0.003 mg ferrocyanide ion/kg bw per day in children and adolescents in the refined exposure scenario.

The Panel considered that the uncertainties identified indicate an overestimation of the exposure to ferrocyanides (E 535–538) as food additives.

Considering that:

- in the refined exposure scenario estimated exposure to ferrocyanides (E 535–538) would correspond approximately to 0.003 mg ferrocyanide ion/kg bw per day in children and adolescents;
- absorption of ferrocyanides from the gastrointestinal tract was low, and there is no accumulation in human;
- ferrocyanides are of low acute toxicity and not mutagenic or carcinogenic;
- reproductive studies were not available, but a NOAEL of 1,000 mg sodium ferrocyanide/kg bw per day (highest dose tested) was identified from a prenatal developmental toxicity study;
- the kidney is the target organ for ferrocyanides toxicity as characterised by the high number of cells excreted in the urine in rats;
- 4.4 mg sodium ferrocyanide/kg bw per day was identified as the NOAEL for this effect in a chronic (2-year) study in rats;
- assuming that the toxicity of this compound is due to the ferrocyanide ion only, the Panel established a ADI for ferrocyanide ion of 0.03 mg/kg bw per day;
- ferrocyanides (E 535–538) are only permitted as food additives in two food categories.

The Panel concluded that ferrocyanides (E 535–538) are of no safety concern in these current authorised use and use levels.

The Panel further concluded that the available data give reason to revise the ADI of 0.025 mg sodium ferrocyanide/kg bw per day (equivalent approximately to 0.02 mg ferrocyanide ion/kg bw per day) based on a subchronic study, to a group ADI for sodium, potassium and calcium ferrocyanide of 0.03 mg/kg bw per day expressed as ferrocyanide ion.

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1. Introduction

The present opinion deals with the re-evaluation of sodium ferrocyanide (E 535), potassium ferrocyanide (E 536), and the evaluation of calcium ferrocyanide (E 538) when used as food additives.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Regulation (EC) No 1333/2008[2] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[3]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[4] of 2001. The report 'Food additives in Europe 2000[5]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.2. Information on existing authorisations and evaluations

Sodium, potassium and calcium ferrocyanides (E 535, E 536 and E 538) are authorised as food additives in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

In EU, sodium and potassium ferrocyanide, used as food additives, was previously evaluated by the Scientific Committee on Food (SCF) in 1990 (SCF, 1991). In that evaluation, the SCF agreed with the acceptable daily intake (ADI) of 0.025 mg/kg body weight (bw) per day (calculated as sodium ferrocyanide) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for sodium and potassium ferrocyanide. The SCF also concluded that 'When used as a processing aid in

[2] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[3] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.
[4] COM(2001) 542 final.
[5] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

the production of wine only small residues are found, and only small technological levels are needed as anticaking agent in salt. Therefore, the Committee has no objection, on toxicological grounds, to the continued use for these purposes'. The Panel noted that in the SCF (1991) evaluation, calcium ferrocyanide was not explicitly mentioned.

The Panel noted that the ADI of 0.025 mg/kg bw per day for sodium and potassium ferrocyanide has been calculated as sodium ferrocyanide while the maximum permitted level is expressed as anhydrous potassium ferrocyanide.

Sodium, potassium and calcium ferrocyanide were evaluated by JECFA in 1969, 1973 and 1974 (JECFA, 1970a, 1974a, 1975). A temporary acceptance of 0–0.00125 mg/kg bw per day was established in 1969 based on a dietary level of 0.05% sodium ferrocyanide (calculated by JECFA to be equivalent to 25 mg/kg bw per day) not causing toxicological effects in a subchronic rat study (Unpublished study by Oser (1959), as cited by JECFA (1975)). The Panel noted that a large uncertainty factor of 20,000 (25 mg/kg bw divided by 0.00125 mg/kg bw) was used. There is no explanation in the toxicological monograph (JECFA, 1970a) or the technical report (JECFA, 1970b) why this unusually high uncertainty factor was used. In 1973, a temporary ADI of 0–0.025 mg/kg bw per day was established on the basis of the data also available for the previous evaluation in 1969 (JECFA, 1970a). However, metabolic studies in man and if necessary a long-term study in one species were required (JECFA, 1974a,b). In 1974, the temporary ADI of 0–0.025 mg/kg per bw (calculated as sodium ferrocyanide) was confirmed and the request for metabolic studies waived due to the notion that such data would only provide limited additional information and require the use of unwanted high levels of radioactive materials in human subjects. A larger uncertainty factor (1,000) than the generally one employed was used to compensate for the absence of a long-term feeding study (JECFA, 1974c).

Potassium and sodium ferrocyanide were evaluated by the UK Committees on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in 1994 (COT, 1994a). The Committee set a group ADI for ferrocyanides of 0–0.05 mg/kg bw per day based on a NOAEL (the lowest dose tested) in a long-term rat study of 50 mg/kg and an uncertainty factor of 100.

The Scientific Committee for Animal Nutrition (SCAN) evaluated the safety for the target animals, the users, the workers, the consumers and the environment of sodium and potassium ferrocyanide used as anticaking agents (European Commission, 2001). It was concluded that sodium and potassium ferrocyanide in salt for feed use (20, 80 and 100 mg/kg in salt for man, poultry and livestock, respectively) is acceptable in regard to the safety for target animals and human consumers.

Sodium, potassium and calcium ferrocyanide were evaluated by a working group established by the Nordic Council of Ministers in 2000 (TemaNord, 2002). Sodium, potassium and calcium ferrocyanide were not considered to cause a safety problem due to the very small quantities consumed. It was noted that without long-term or reproductive studies a full toxicological evaluation would not be possible.

2. Data and methodologies

Data

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier. EFSA launched public call for data[6] and, if relevant, contacted other risk assessment bodies to collect relevant information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public call for data, information from previous evaluations and additional available literature up to the last Working Group (WG) meeting.[7] Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based however these were not always available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[8]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of sodium ferrocyanide (E 535), potassium ferrocyanide (E 536) and calcium ferrocyanide (E 538) in food products.

[6] Call for Call for technical and toxicological data on miscellaneous food additives to be re-evaluated under the Regulation (EU) No 257/2010. Published: 11 August 2017. Available from: https://www.efsa.europa.eu/en/consultations/call/170811 Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

[7] 11–12 May 2018.

[8] Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The ANS Panel assessed the safety of sodium ferrocyanide (E 535), potassium ferrocyanide (E 536), and calcium ferrocyanide (E 538) as food additives in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a,b,c) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake is expressed as equivalent. When in human studies in adults (aged above 18 years), the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a).

Dietary exposure to sodium ferrocyanide (E 535), potassium ferrocyanide (E 536), and calcium ferrocyanide (E 538) from their use as food additives was estimated combining sodium chloride dietary intake with maximum levels according to Annex II to Regulation (EC) No 1333/2008[9] and reported use levels submitted to EFSA following a call for data. These sodium chloride dietary intakes were calculated from sodium intake which was assessed from urinary excretion studies. These studies were collected through EFSA focal points and the members of the EFSA Food Consumption Network. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substances

In ferrocyanide coordination compounds, iron has a (positive) divalent oxidation state (Fe^{2+}): these complexes have an octahedral geometry characterised by the coordination number of 6 (Figure 1 shows a simplified chemical structure of $K_4[Fe(CN)_6]$ as an example). The hexacyanoferrate(II) anion $[Fe(CN)_6]^{4-}$ – commonly called ferrocyanide (CAS Registry No 13408-63-4) – is very stable because of the strong bonding between iron and each cyanide group. The free ferrocyanic acid $H_4[Fe(CN)_6]$, or tetrahydrogen hexakiscyanoferrate (CAS Registry No 17126-47-5), is a strong tetrabasic acid when dissolved in water (Perrin, 1969; Cotton et al., 1999; Stolzenberg, 2005).

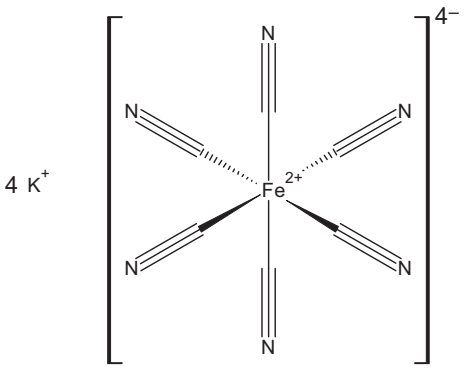

Figure 1: Simplified structural formula of potassium ferrocyanide (anhydrous form)

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

Sodium ferrocyanide (E 535)

According to Commission Regulation (EU) 231/2012[10], sodium ferrocyanide (E 535) has molecular formula $Na_4[Fe(CN)_6] \cdot 10H_2O$, EINECS (EC) No 237-081-9, and molecular weight 484.1 g/mol.

The EINECS (EC) No 237-081-9 corresponds to the CAS Registry No 13601-19-9 which is for the anhydrous form. The CAS Registry number for the hydrate form is 14434-22-1 (these identifiers are not present in the Regulation). In JECFA (2006), the chemical is identified as sodium ferrocyanide with INS No 535; the reported CAS Registry number identifies the anhydrous form.

Based mainly on Commission Regulation (EU) 231/2012, JECFA (2006), and SciFinder Online, a selection of synonyms and identifiers includes yellow prussiate of soda; sodium hexacyanoferrate; hexacyanoferrate of sodium; sodium hexacyanoferrate decahydrate; tetrasodium hexacyanoferrate(4–) decahydrate.

Potassium ferrocyanide (E 536)

According to Commission Regulation (EU) 231/2012, potassium ferrocyanide (E 536) has molecular formula $K_4[Fe(CN)_6] \cdot 3H_2O$, EINECS (EC) No 237-722-2, and molecular weight 422.4 g/mol.

The EINECS (EC) No 237-722-2 correspond to the CAS Registry No 13943-58-3 which is for the anhydrous form. The CAS Registry number for the hydrate form is 14459-95-1 (these identifiers are not present in the Regulation). In JECFA (2006), the chemical is identified as potassium ferrocyanide with INS No 536; the reported CAS Registry number identifies the anhydrous form.

Based mainly on Commission Regulation (EU) 231/2012, JECFA (2006), and SciFinder Online, a selection of synonyms and identifiers includes: yellow prussiate of potash; potassium hexacyanoferrate; hexacyanoferrate of potassium; potassium ferrocyanide trihydrate; tetrapotassium hexacyanoferrate(4–) trihydrate.

Calcium ferrocyanide (E 538)

According to Commission Regulation (EU) 231/2012, calcium ferrocyanide (E 538) has molecular formula $Ca_2[Fe(CN)_6] \cdot 12H_2O$ and molecular weight 508.3 g/mol. In JECFA (2006), the chemical is identified as calcium ferrocyanide with INS No 538. The Panel noted that, according to the database (Scifinder Online and EC Inventory of Chemicals), the EINECS (EC) No 215-476-7 and the CAS Registry No 1327-39-5 – respectively reported in the Regulation and in JECFA (2006) – refer to calcium aluminium silicate and not to calcium ferrocyanide (E 538).

The EINECS (EC) No 237-508-9 and the CAS Registry No 13821-08-4 correspond to the calcium ferrocyanide anhydrous.

Based mainly on Commission Regulation (EU) 231/2012, JECFA (2006) and SciFinder Online, a selection of synonyms and identifiers includes: yellow prussiate of lime; calcium hexacyanoferrate; hexacyanoferrate of calcium; dicalcium hexacyanoferrate(4–).

The CAS Registry and EINECS (EC) numbers reported above for the three ferrocyanides were subject to confirmatory steps to minimise the uncertainty of an equivocal identification. However, in addition to the observations brought forward for calcium ferrocyanide, the Panel also noted that the same CAS Registry and/or EINECS (EC) numbers may occasionally be found to identify marketed hydrous or anhydrous compounds.

3.1.2. Specifications

Commission Regulation (EU) No 231/2012 lays down specifications for sodium ferrocyanide (E 535), potassium ferrocyanide (E 536), and calcium ferrocyanide (E 538) used as food additives: as the three chemicals have substantially the same specifications, the latter have been reported in Table 1 only once although in the Regulation they come in individual sections. JECFA also established specifications for the same chemicals to be used as food additives (JECFA, 2006), as shown in Table 1.

[10] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1.

Table 1: Specifications established for sodium ferrocyanide (E 535), potassium ferrocyanide (E 536), and calcium ferrocyanide (E 538) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Assay	Content not less than 99.0% of the respective ferrocyanide	Not less than 99.0% of the respective ferrocyanide
Description	Yellow crystals or crystalline powder for sodium and calcium ferrocyanides; lemon yellow crystals for potassium ferrocyanide	Yellow crystals or crystalline powder
Identification		
Test for metal (Na, K, or Ca)	Passes test of the respective ferrocyanide	Passes test of the respective ferrocyanide
Test for ferrocyanide	Passes test	Passes test
Solubility	–	All soluble in water; sodium and potassium ferrocyanides insoluble in ethanol
Purity		
Free moisture	Not more than 1.0%	–
Water insoluble matter	Not more than 0.03%	–
Chloride	Not more than 0.2%	–
Sulfate	Not more than 0.1%	–
Free cyanide	Not detectable	Not detectable
Ferricyanide	Not detectable	Not detectable
Lead	Not more than 5 mg/kg	Not more than 5 mg/kg
Arsenic	–	Not more than 3 mg/kg

The Panel noted that the solubility and the limit for arsenic are not specified in the EU specification in contrast to the JECFA specification (2006).

3.1.3. Manufacturing process

As reported by Wong-Chong and co-workers (2006), the three food additives described above are fully synthetic. Sodium ferrocyanide is produced in aqueous medium from crude sodium cyanide and ferrous sulphate according to the canonical expression:

$$6NaCN + FeSO_4 + Heat \rightarrow Na_4[Fe(CN)_6] + Na_2SO_4$$

The sodium ferrocyanide decahydrate salt is recovered by crystallisation. The potassium salt is produced by reacting sodium ferrocyanide with calcium hydroxide and potassium chloride and carbonate according to the following reactions:

$$Na_4[Fe(CN)_6] + 2Ca(OH)_2 \rightarrow Ca_2[Fe(CN)_6] + 4Na(OH)$$

$$Ca_2[Fe(CN)_6] + 2K_2CO_3 \rightarrow K_4[Fe(CN)_6] + CaCO_3$$

Calcium ferrocyanide is produced by reacting sodium ferrocyanide with calcium hydroxide, as visible above.

According to Stolzenberg (2005), $Na4[Fe(CN)_6]\cdot 10H_2O$ is produced from calcium cyanide, iron(II) sulphate, and sodium carbonate in aqueous medium at 100°C, with a process similar to that described hereafter for the potassium derivative. $K_4[Fe(CN)_6]\cdot 3H_2O$ is prepared from calcium cyanide and iron(II) sulfate at a temperature above 100°C; insoluble products are removed and potassium chloride is added; the produced precipitate of calcium potassium ferrocyanide is redissolved as the potassium salt by addition of potassium carbonate; the insoluble calcium carbonate is removed and $K_4[Fe(CN)_6]\cdot 3H_2O$ is crystallised by rapid cooling. $Ca_2[Fe(CN)_6]$ is obtained by reaction of liquid or gaseous hydrogen

cyanide with iron(II) chloride in an alkaline aqueous medium (pH > 8) containing calcium hydroxide or calcium carbonate.

3.1.4. Methods of analysis in food

During the course of the long chemical history of ferrocyanides, the latter have found many diverse applications in analytical chemistry; likewise, many analytical methods have been developed for their detection in various matrices.

In the paper by Roberts and Wilson (1968), ferrocyanide ($[Fe(CN)_6]^{4-}$) in commercial sodium chloride was determined spectrophotometrically as its iron complex in the range 0.013–50.0 mg/kg. The iron complex was concentrated from a large volume of sample solution by filtration on kieselguhr, and a reproducible Prussian Blue colour formed in a small volume under controlled conditions. Aquopentacyanoferrate ($[Fe(CN)_5H_2O]^{3-}$), a possible albeit quite uncommon interfering substance, could be determined simultaneously, and the amounts of each complex present were thereby estimated. Some interference was caused by carbonyl pentacyanoferrate ($[Fe(CN)_5CO]^{3-}$), a compound unusually present whose precise determination was however achieved by using a similar principle of concentration, but with different reagents to develop the iron complex. The optical densities were measured at 700 nm for ferrocyanide, at 700 and 860 nm for aquopentacyanoferrate, and at 530 nm for carbonyl pentacyanoferrate. The procedure described in general exhibited quantitative recoveries and was suitable for ferrocyanide determination at concentrations as low as 0.10 mg/kg salt. No interference was caused by the presence of other iron-cyanogen complexes, or by the usual impurities and additives in commercial salts.

A rapid method was developed by Li et al. (2006) for the determination of trace amounts of potassium ferrocyanide ($K_4[Fe(CN)_6]$) in salted foods (eggplants) and table salt. When potassium ferrocyanide reacted with triaminotriphenylmethane dyes to form ion-association complexes, resonance Rayleigh scattering (RRS) intensities were enhanced greatly relative to the uncomplexed chemicals. Experimental trials were carried out with ethyl violet (EV), crystal violet (CV), and methyl violet (MV), the highest RRS response being obtained with EV. A spectrofluorophotometer was used for recording the RRS spectra and measuring the scattered intensity; the maximum peaks occurred at approximately 329 nm. The detection limit of the EV system was 7.8 ng/mL over the 4.8–6.8-pH range. The method showed a quantitative recovery for potassium ferrocyanide at mg/kg levels (RSD < 5%) and was considered to be suitable for the determination of trace amounts of potassium ferrocyanide in colour salted food. In a subsequent paper – also focusing on the determination of potassium ferrocyanide in salted food (eggplants and lavers) and table salt – Li et al. (2007) reported that double-charged triaminotriphenylmethane dyes (e.g. methyl green (MeG), iodine green (IG)) in acidic medium (pH 1.0) reacted with the ferrocyanide anion to form 2:1 ion-association complexes. The latter were characterised by a change of absorption and a remarkable enhancement of RRS intensities relative to the uncomplexed chemicals. The maximum RRS wavelengths were all located at 276 nm; a spectrofluorophotometer was used for recording the RRS spectra and measuring the scattering intensity. The intensity of RRS was directly proportional to the concentration of the ferrocyanide anion in the ranges of 0.03–5.7 and 0.04–5.9 μg/mL for the MeG and IG systems, respectively. The RRS method showed a good selectivity and high sensitivity, with detection limits for potassium ferrocyanide of 9.3 and 11.2 ng/mL for the MeG and IG systems, respectively. In salted eggplant and laver samples, potassium ferrocyanide recovery was quantitative at the levels tested (low mg/kg) (RSD = 3.2–6.2%).

A flow injection (FI) system for a sensitive determination of ferrocyanide was described by Yamane et al. (2006). The anion exchange column incorporated in the FI system was utilised for separation and preconcentration of ferrocyanide from a large excess of sodium chloride (matrix) and co-existing other substances, and for the detection reaction of ferrocyanide, adsorbed on the column, with Fe(III) complex with 1,10-phenanthroline ($[Fe(o\text{-phen})_3]^{3+}$): the resultant ferroin ($[Fe(o\text{-phen})_3]^{2+}$) was detected spectrophotometrically at 512 nm. The Fe(III) 1,10-phenanthroline complex was prepared in-line by passing a ferroin solution through a manganese dioxide reactor in the flow system. A linear ferrocyanide calibration over the range of 0–0.3 mg/kg in the presence of sodium chloride (0.5 mol/L) was obtained using a 6-m sample loop injection. The coefficient of variation for (potassium) ferrocyanide added to purified salt in the range of 0.050–0.200 mg/kg was better than 5%; the estimated limit of detection was 0.003 mg/kg. The FI system was successfully applied to determine ferrocyanide at mg/kg level in real salt samples with a precision better than 3% and quantitative recovery.

Lim et al. (2018) developed and validated a rapid high-performance liquid chromatography (HPLC) method to determine the presence of ferrocyanide ions ($[Fe(CN)_6]^{4-}$) in food grade salts (sodium chloride). An analytical column coupled with a guard column and mobile phase comprised of sodium perchlorate and sodium hydroxide were employed with a photodiode array detector set at a wavelength of 221 nm. Samples were dissolved in 0.02 M sodium hydroxide solution and filtered through a 0.22-μm polyvinylidene difluoride membrane. For processed salts including herbs and spices, a C_{18} cartridge was applied to minimise interference from salt matrices. The method was characterised as to linearity, accuracy (recovery), precision, limit of detection (LOD) and limit of quantification (LOQ), and measurement uncertainty. Linearity was good from 0.1 to 10 mg/L; LOD and LOQ values were determined to be 0.02 and 0.07 mg/kg, respectively. Ferrocyanide recoveries in six salt matrices – originally ferrocyanide-free, then each spiked with (sodium) ferrocyanide at 1, 5, and 10 mg/kg for the validation study – ranged from 80.3% to 103.5% (RSD = 0.3–4.4%). The method was applied to a large number of commercial products. These results indicated that the method was suitable for ferrocyanide ion determination in various food grade salts, with a good potential for application to routine analysis.

3.1.5. Stability of the substance, and reaction and fate in food

Kruse and Thibault (1973) investigated the decomposition of ferro- and ferricyanide ($K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$) as a function of pH, illumination, and temperature. For routine measurements of hydrogen cyanide (HCN), the separation of the free acid from the sample by means of diffusion in Conway microdiffusion cells was employed; the final measurements were carried out by titration or colorimetry. Experiments were conducted to measure the transport of hydrogen cyanide as a function of pH and time: complete recovery of free cyanide was obtained at pH 7 or lower, in diffusion periods of ~ 5 h. At pH higher than 9, recovery decreased; at pH below 5, the decomposition of complex cyanides was more significant. Complex cyanides decomposed only very slowly, if at all, above pH 5 in the dark; however, the normal tungsten or fluorescent light and lower pH greatly accelerated the decomposition.

Storage life of meat and meat products is often limited by oxidative processes (such as colour changes from red to brown/grey and/or development of rancid taste); therefore, factors influencing oxidative changes are of great interest to meat manufacturers. Influence of salt (NaCl) and potassium ferrocyanide ($K_4[Fe(CN)_6]$) on oxidative stability of minced pork meat was investigated by Hansen et al. (1996). Ferrocyanide was found to affect lipid oxidation in the frozen (−22°C) meat both in usual concentrations (≤ 0.4 mg/kg meat), when added together with food grade salt to yield 2% salt in the product, and in unusually high concentrations (≥ 80 mg/kg meat) added separately or together with chemically pure sodium chloride. The level of ferrocyanide obtained from adding 2% salt accelerated the development of lipid hydroperoxides, but affected the development of thiobarbituric acid-reactive substances (TBARS) to a lesser degree; high levels of ferrocyanide seemed to protect hydroperoxides from degradation to secondary lipid oxidation products (measured as TBARS). Addition of ferrocyanide in high concentrations resulted in immediate discoloration of the meat independent of the presence of 2% salt, whereas products with added commercial table salt (ferrocyanide at level of 7 mg/kg salt), products with added pure sodium chloride, and products without additives did not discolour immediately. However, after approximately 3 weeks of storage all products discoloured at a similar rate possibly due to a relevant contribution from background colour, reflecting colour changes just below the surface of products. Products with high concentrations of ferrocyanide were observed to have become red within the product. Products added the highest ferrocyanide concentration (17,500 mg/kg meat) became redder at the surface between the first and third week of storage (overall storage duration, 56 days). This was thought to support the suggestion that ferrocyanide was oxidised to ferricyanide ($[Fe(CN)_6]^{3-}$) during storage under the experimental conditions adopted, as ferricyanide was a red complex and could contribute to the colour of the product. A mechanism involving the ferrocyanide-ferricyanide redox couple of pigment-catalyzed lipid oxidation was suggested, based on an observed correlation between oxymyoglobin oxidation (measured as tristimulus colorimetry) and lipid oxidation (measured as TBARS).

Nguyen et al. (2012) investigated the effects of added potassium ferrocyanide ($K_4[Fe(CN)_6]$) in different concentrations (2.5, 7.5, and 100 mg/kg) in salt on lipid oxidation in cod during salting, storage (up to 6 months, at ~ 2°C), and rehydration. An increase in ferrocyanide concentration accelerated lipid oxidation of the salted cod, as observed by increases in lipid hydroperoxides (PV) and TBARS, as well as in the development of fluorescence compounds: the fluorescence shift (δF) was determined in both the organic-chloroform phase (δF_{OR}) and the aqueous-methanol phase (δF_{AQ}) from

extraction processes. A yellow discolouration (higher b* value, b* being an indicator of yellowness) of salted cod was associated with higher levels of oxidation derivatives. High correlations were found between PV, TBARS and free fatty acids (FFA), as well as between FFA and δF_{OR}. The results of principal component analysis showed that TBARS, b* value and δF_{OR} were the strongest indicators of lipid oxidation during salting and storage.

Dorazio and Bruckner (2015) presented findings on the mode of action of submonoatomic layers of sodium ferrocyanide on sodium chloride crystals, to act as an anticaking agent through nucleation inhibition. Sodium ferrocyanide on store-bought table salt could be readily detected due to the appearance of an intense blue-green colour following formation of Prussian Blue ($Fe_4[Fe(CN)_6]_3 \cdot nH_2O$) upon addition of slightly yellow aqueous iron(III) trichloride.

3.2. Authorised uses and use levels

Maximum levels of Ferrocyanides (E 535–538) have been defined in Annex II to Regulation (EC) No 1333/2008[11] on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, ferrocyanides (E 535–538) are authorised food additives in the EU at 20 mg/kg in 2 categories listed in Table 2.

Table 2: MPLs of ferrocyanides (E 535–538) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/ group	Restrictions/ exception	MPL (mg/L or mg/kg as appropriate)
12.1.1	Salt	E 535–538[(a)]		20[(b)]
12.1.2	Salt substitutes	E 535–538[(a)]		20[(b)]

MPL: maximum permitted level.
(a): The additives may be added individually or in combination.
(b): The maximum level is expressed as anhydrous potassium ferrocyanide.

Ferrocyanides (E 535–538) are not authorised according to Annex III to Regulation (EC) No 1333/2008.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of ferrocyanides (E 535–538)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call,[12] for occurrence data (usage level and/or concentration data) on ferrocyanides (E 535–538). In response to this public call, updated information on the actual use levels of ferrocyanides (E 535–538) in foods was made available to EFSA by industry. No analytical data on the concentration of ferrocyanides (E 535–538) in foods were made available by the Member States.

Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 16) of ferrocyanides (E 535–538) in salt (FC 12.1.1) and in other foods containing salt (n = 62), and thus containing ferrocyanides (E 535–538) as carry over, covering 11 food categories.

Updated information on the actual use levels of ferrocyanides (E 535–538) in foods was made available to EFSA by FoodDrinkEurope (FDE, Documentation provided to EFSA No. 3), European Potato Processors' Association (EUPPA, Documentation provided to EFSA No. 4), European Salt Producers' Association (EU_SALT, Documentation provided to EFSA No. 5), Ornua (Documentation provided to EFSA No. 6) and Intersnack (Documentation provided to EFSA No. 7).

[11] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.
[12] https://www.efsa.europa.eu/en/data/call/170223

Appendix A provides data on the use levels of ferrocyanides (E 535–538) in foods as reported by industry.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 2.5 million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.

For the purpose of this Scientific Opinion, the Mintel's GNPD was used for checking the labelling of food and beverages products and food supplements for ferrocyanides (E 535–538) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to the Mintel's GNPD, ferrocyanides (E 535–538) were labelled on 399 products between January 2013 and April 2018.

Appendix B lists the percentage of the food products labelled with ferrocyanides (E 535–538) out of the total number of food products per food subcategories according to the Mintel's GNPD food classification. The percentages ranged from less than 0.1% in many food subcategories to 2.1% in the Mintel's GNPD food subcategory 'Seasonings' which includes products falling under legislation categories FCs 12.1.1 Salt and 12.1.2 Salt substitutes. Taking into account only the salt products from the sub-category 'Seasonings' (n = 1,533), 13% of them contained ferrocyanides (E 535–538).

All other subcategories presented in the Mintel's GNPD may contain ferrocyanides (E 535–538) as a carry-over from salt.

Considering the individual E numbers of ferrocyanides (E 535–538), the majority of the products were labelled with sodium ferrocyanide (E 535) (n = 305), whereas 101 products were labelled with potassium ferrocyanide (E 536). Some foods were labelled with a combination of both additives. No products were labelled with calcium ferrocyanide (E 538).

3.3.3. Salt intake data used for exposure assessment to ferrocyanides (E 535–538)

Ferrocyanides (E 535–538) are solely authorised in FCs 12.1.1 Salt and 12.1.2 Salt substitutes, it is therefore important to accurately assess the intake of salt to estimate their exposure. However, there are considerable challenges to accurately measure the usual salt intake in individuals (McLean et al., 2017). Dietary surveys are commonly not considered as a good source of information because a significant part of the salt intake is coming from the consumption of processed foods and their salt content is highly variable over time and food types so there is a high uncertainty using food composition tables.

Another issue is that people add salts to their food (e.g. during preparation or while eating). This part of the salt intake is not well covered in dietary surveys.

A typical way of estimation of the salt intake is its calculation from the urinary excretion of sodium. Urinary sodium excretion has traditionally been used as a biomarker of sodium intake (Gibson, 2005; Freedman et al., 2015), as it is considered to be more accurate than estimates of intake based on dietary assessments. Twenty-four-hour urinary sodium excretion is used as a measure of average sodium intake at the population level (WHO, 2011). In healthy people, almost all dietary sodium intake is absorbed. Urine is the major route of sodium excretion with mean recovery rates of dietary sodium in the urine generally ranging from 80% to 95%.

The ANS Panel decided to use estimated salt intake data from urinary excretion studies of sodium for the assessment of exposure to ferrocyanides (E 535–538) instead of the food consumption data from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) which are used in the other opinions related to the re-evaluation of food additives.

FC 12.1.2 salts substitutes which are not sodium-based (e.g. potassium chlorides) are only partly taken into account in the current estimates of ferrocyanides and this can lead to an underestimation.

The NDA Panel drafted an opinion[13] on dietary reference values for sodium and submitted it to a public consultation in 2017. The draft opinion describes that in 2016 an overview of sodium intake in European populations was prepared based on data on sodium urinary excretion in European populations

[13] https://www.efsa.europa.eu/en/consultations/call/170929

collected through EFSA focal points and the members of the EFSA Food Consumption Network. Data were received from 17 countries, and the most recent surveys, conducted between 2002 and 2014, were selected. Three countries provided urinary sodium excretion data in children (Austria, Iceland, Spain) and 16 countries provided urinary sodium excretion data in adults (Austria, Belgium, Croatia, the Czech republic, Finland, Germany, Greece, Hungary, Ireland, Norway, Slovenia, Spain, Sweden, Switzerland, the Netherlands and the United Kingdom). The majority of countries used 24-h urine collection, while three countries collected spot or timed urine collection and estimated daily sodium excretion through arithmetic extrapolation. Studies using 24-h urine collection were heterogeneous with respect to the methods and criteria applied for the assessment and exclusion of incomplete or unreliable urine collection (e.g. PABA recovery, creatinine excretion levels, urinary volume, self-reporting of incomplete samples). Some studies were designed as national monitoring surveys, while others were conducted as part of broader observational studies. Samples sizes also varied widely, from tens to thousands of people.

The NDA Panel noted that a single 24-h urine collection does not reliably reflect an individual's usual intake, primarily due to within person day-to-day variability in sodium intake and excretion. The Panel therefore considered that a single 24-h collection can be used to estimate average group sodium intakes, but can lead to random misclassification of study participants in relation to their usual sodium intake. In addition, the Panel noted that incomplete 24-h urine collections could have introduced bias in intake estimates.

More convenient methods such as casual spot and timed spot urine collections (i.e. collection during the day, evening, or overnight) have also been used as indicators of sodium intake. Day-to-day and diurnal variations in sodium excretion render these measures highly variable at the individual level; hence, these methods are subject to greater within-person variability in sodium excretion than 24-h urine collections (Ji et al., 2014; Wang et al., 2013; Sun et al., 2017). Predictive equations have been developed to estimate 24-h urinary sodium excretion from spot urine samples (Kawasaki et al., 1993; Tanaka et al., 2002; Brown et al., 2013).

The NDA Panel noted that both overnight and spot urine collections are easier for participants, but their reliability to estimate daily sodium intake is largely affected by circadian variations in individual sodium excretion. The NDA Panel further noted that estimates of individual daily intakes from predictive equations based on spot urine samples can be biased, particularly at the lower and higher ends of the distribution and are therefore unreliable.

The ANS Panel decided to take into account all surveys from the NDA opinion in the current assessment. Data on surveys and methodologies used for the estimation of salt intake and the exposure assessment of ferrocyanides (E 535–538) are presented in Appendix C. Sodium chloride intake (NaCl g/day (Y)) was calculated from the sodium excreted in urine (mmol Na/day (X)) reported in the publications with the following equation:

$$Y = [(X \times 22.99)/0.4)]/1,000.$$

Based on the fact that 22.99 g sodium equals to 1 mole of sodium, and 1 g of salt contains 0.4 g sodium and 0.6 g chloride.

3.4. Exposure estimate

3.4.1. Exposure to ferrocyanides (E 535–538) from their use as food additives

The Panel estimated chronic exposure to ferrocyanides (E 535–538) for children and adolescents (boys and girls at the age of 6–18), adults and the elderly (men and women at the age of 18–79) for different Member States. Dietary exposure to ferrocyanides (E 535–538) was calculated by multiplying ferrocyanides (E 535–538) concentrations with all estimated salt consumption amounts reported in Appendix C, including the mean and high (up to the 75th percentile) consumption. Exposure estimates per kg body weight were obtained by using the standard body weight for each age group (EFSA, 2012).

Exposure to ferrocyanides (E 535–538) was estimated by the ANS Panel based on two different sets of concentration data: (1) MPL as set down for FC 12.1.1 Salt in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*); and (2) mean use level reported by industry for FC 12.1.1. Salt of 9.7 mg/kg (defined as the *refined exposure assessment scenario*). The Panel noted that the highest reported use level of ferrocyanides (E 535–538) in salt was equal to the MPL.

Dietary exposure to ferrocyanides (E 535–538)

Table 3 summarises the estimated exposure to ferrocyanides (E 535–538) from their use as food additives in the population groups according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix D.

Dietary exposure to ferrocyanides was calculated based on mean and high levels consumption of salts.

Table 3: Summary of dietary exposure to ferrocyanides (E 535–538) from their use as food additives in the maximum level exposure assessment scenario and in the refined exposure scenario, mg anhydrous potassium ferrocyanide/kg bw per day

	Children and adolescents (6–18 years)		Adults and elderly (18–79 years)	
	Min	Max	Min	Max
MPL scenario				
Mean	0.003	0.007	0.002	0.004
High[(a)]	0.004	0.009	0.002	0.005
Refined scenario				
Mean	0.002	0.004	0.001	0.002
High[(a)]	0.002	0.004	0.001	0.003

(a): High levels up to p75 (see Appendix C).

In the *regulatory maximum level exposure assessment scenario*, based on the mean consumption of salt, the exposure to ferrocyanides (E 535–538) from their use as a food additive ranged from 0.002 mg/kg bw per day in adults and elderly to 0.007 mg/kg bw per day in children and adolescents. For the high consumers of salt, exposure in the same scenario ranged from 0.002 mg/kg bw per day in adults and elderly to 0.009 mg/kg bw per day in children and adolescents.

In the *refined estimated exposure scenario, based on* with the mean consumption of salt the exposure ranged from 0.001 mg/kg bw per day in adults and elderly to 0.004 mg/kg bw per day in children and adolescents. For the high consumers of salt, exposure was in the same ranges.

Uncertainty analysis

Uncertainties in the exposure assessment of ferrocyanides (E 535–538) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 4.

Table 4: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Use of urinary excretion studies for the assessment of salt intake	+/–
Sodium intake data: different methodologies/representativeness	+/–
Sodium intake does not only originate from salt	+
Use of standard body weights for the exposure assessment	+/–
Assumption that all salt contains the additives while the Mintel's GNPD indicates that only 13% of the salt products in the database contain ferrocyanides (E 535 and E 536)	+
Salt substitutes partly considered in the exposure assessment (only their sodium content is taken into account)	–
Regulatory maximum level exposure assessment scenario: • exposure calculations based on the MPL according to Annex II to Regulation (EC) No 1333/2008	+
Refined exposure assessment scenarios: • exposure calculations based on the mean levels (reported use from industries)	+/–
Assumption that reported use levels were expressed as anhydrous ferrocyanide salts	+

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified indicate an overestimation of the exposure to ferrocyanides (E 535–538) as food additives in European countries for both the regulatory maximum level and the refined exposure scenario.

3.5. Biological and Toxicological data

No toxicological information was submitted for the re-evaluation of sodium, potassium and calcium ferrocyanide following an EFSA public call for data, prior to the start of this re-evaluation.

3.5.1. Absorption, distribution, metabolism and excretion

The relevant studies evaluated by JECFA (1974a, 1975), as well as the additional studies are summarised below.

Rats were dosed orally with 200 mg/kg bw potassium ferrocyanide. Approximately 47% was reported to have been excreted unchanged in the faeces and 3% in the urine. Faecal and urinary excretion of ferrocyanide and thiocyanate was at a maximum from day 1 to 3 after dosing and thereafter declined to a low level (unpublished data from Gage (1950), cited in JECFA (1975).

Female Wistar rats (250–280 g, 3 animals) were administered ^{59}Fe- and ^{14}C dual-labelled potassium ferrocyanide as $K_4[^{59}Fe(^{14}CN)_6]\cdot 3H_2O$ in a single dose of 10 mg per animal by gastric intubation (Nielsen et al., 1990a). Urine and faeces were collected for 7 days following administration. ^{14}C was measured in expired air during the first 24 h after administration. Whole body retention (WBR) of ^{59}Fe was measured after 7 days and ^{59}Fe and ^{14}C was measured in blood, liver, spleen, kidneys, heart/lung, gut and carcass after 7–10 days. Total ^{59}Fe-activity (mean ± SD) in faeces and urine after 7 days was 94.4 ± 2.9% and 2.5 ± 0.8% of dose, respectively. The total absorption of potassium ferrocyanide was calculated by the authors to be up to 5.6% (based on subtracting percentage ^{59}Fe-activity in faeces from 100% of dose). WBR and total recovery of ^{59}Fe (mean ± SD) was 0.09 ± 2.1 and 97 ± 2.1% of the dose, respectively. The erythrocyte incorporation (mean ± SD) of ^{59}Fe was 0.005 ± 0.0007% of the dose. Total ^{14}C-activity (mean ± SD) in urine after 7 days was 2.8 ± 0.5% of dose. ^{59}Fe-activity was detected in liver, spleen, kidney and heart/lungs. The amount of expired ^{14}C (mean ± SD) during 24 h after gastric intubation was 0.04 ± 0.01% of administered dose. The authors estimated that less than 0.06 mg free cyanide/kg bw was absorbed after oral administration of 10 mg ^{59}Fe- and ^{14}C-labelled potassium ferrocyanide.

Likewise, following an oral dose of 500 mg (6.2–7.1 mg/kg-bw) of potassium ferric ferrocyanide in humans (n = 3, male), absorption was 0.25%–0.42% of the Fe^{II} and Fe^{III}, respectively, and whole body retention after seven days was 0.03–0.07% (Nielsen et al., 1990b). Elimination was > 97% and > 99% faecal in rats and humans, respectively.

Overall, potassium ferrocyanide was absorbed to a limited extent from the gastrointestinal tract following oral administration to rats (unpublished data from Gage (1950), cited in JECFA (1975); Nielsen et al., 1990a). The majority was excreted unchanged in the faeces (approximately 95% of the dose). Ferrocyanide was detected in liver, spleen, kidney, heart and lungs. Free iron was detected in erythrocytes and free cyanide was detected in urine and expired air. The exposure to free cyanide was estimated to be less than 0.06 mg/kg bw after oral administration to rats of 10 mg ^{59}Fe- and ^{14}C-labelled potassium ferrocyanide per animal (Nielsen et al., 1990a). In humans, absorption was 0.25–0.42% (Nielsen et al., 1990b).

3.5.2. Acute toxicity

The oral LD_{50} value for potassium ferrocyanide was reported to be between 1600 and 3,200 mg/kg bw in rats (unpublished data by Fassett cited in JECFA (1975)).

3.5.3. Short-term and subchronic toxicity

Rats

Rats (10 animals/sex per group) were given 0%, 0.05%, 0.5% or 5% sodium ferrocyanide in the diet for 13 weeks (equivalent to 0, 45, 450 and 4,500 mg/kg bw per day using the EFSA default value of 0.09 for subchronic studies (EFSA, 2012)) (unpublished study by Oser (1959), cited in JECFA (1975)) Slightly lower growth rate and food consumption was observed at the 5% level. Haematocrit and haemoglobin values were lower at the 5% level. Kidney weight was higher at the 5% level (both sexes) and at the 0.5% level (females). Male adrenal and female pituitary gland weights were higher

at the 5% level. A minimal degree of tubular damage in the kidneys was observed in rats at the 0.5% level. The effect was more marked at the 5% level where granular and calcified deposits were observed The Panel noted that the ADI set by JECFA for sodium, potassium and calcium ferrocyanide was based on this study. No further details included in the JECFA evaluation.

Dogs

Beagles (4 animals/sex per group) were given 0, 10, 100 or 1,000 mg/kg sodium ferrocyanide in the diet (equivalent to 0, 0.25, 2.5 and 25 mg/kg bw per day) for 13 weeks (unpublished data from Morgaridge (1970), cited in JECFA (1975)). No treatment-related effects were observed in appearance, behaviour, body weight change, physical condition, urinary pathology, haematology, biochemical parameters, or gross and histopathology.

Overall, treatment-related effects were observed in kidneys (higher organ weight, tubular damage and granular and calcified deposits) in rats given 0.5% and 5% sodium ferrocyanide (450 and 4,500 mg/kg bw per day) in the diet for 13 weeks (unpublished study by Oser (1959), cited in JECFA (1975)). No treatment-related effects were observed in dogs given dietary sodium ferrocyanide up to 1,000 mg/kg (25 mg/kg bw per day) for 13 weeks (Unpublished data from Morgaridge (1970), cited in JECFA (1975).

3.5.4. Genotoxicity

No data were submitted to EFSA following a public call for data. Additional data were identified in the literature search and are summarised below. Neither the SCF (1991) nor JECFA (1970a, 1974a, 1975) have described any data on genotoxicity of sodium, potassium or calcium ferrocyanide.

Potassium ferrocyanide was reported to be negative at a concentration of 2.5 mM when tested for mutagenicity in a Rec-assay system with *Bacillus subtilis* strains H17 and M45 (Nishioka, 1975).

Potassium ferrocyanide was reported to be negative at concentrations ranging from 5 to 500 mM when tested for mutagenicity in a Rec-assay system with *B. subtilis* strains H17 and M45 (Kanematsu et al., 1980).

Potassium ferrocyanide was reported to be negative at concentrations ranging from 1 to 10,000 nM/mL when tested for genotoxic potential in the SOS Chromotest using *Escherichia coli* strains PQ37 and PQ35 with or without metabolic activation (Olivier and Marzin, 1987).

Sodium and potassium ferrocyanide were tested for genotoxicity in human lymphocyte cells in an *in vitro* Comet assay (Basu et al., 2013). The compounds were tested at concentrations of 0, 1, 5 and 10 mM for 3 h. The tail DNA (%) ($\pm$ SEM) was increased after treatment with potassium ferrocyanide at 5 mM (3.55 $\pm$ 0.12) and 10 mM (4.22 $\pm$ 0.5) mM when compared to control (2.16 $\pm$ 0.15) ($p \leq 0.05$). Sodium ferrocyanide did not induce DNA damage. Potassium ferrocyanide significantly reduced cell viability at all tested concentrations for about 20% compared to control, whereas sodium ferrocyanide significantly reduced cell viability for approximately 17% at the highest tested concentration only (10 mM).

In summary, potassium ferrocyanide did not show a mutagenic potential in two Rec assays with *B. subtilis* strains H17 and M45 in concentrations up to 500 mM (Nishioka, 1975, Kanematsu et al., 1980) or a genotoxic potential in the SOS Chromotest using *E. coli* strains PQ37 and PQ35 at in concentrations up to 10 mM Olivier and Marzin, 1987). Increased DNA damage was reported in an *in vitro* indicator assay with potassium but not sodium ferrocyanide at high doses. The Panel noted that the effect may be related to an indirect mechanism, such as ROS generation under *in vitro* conditions, which is based on the evidence that in food systems potassium ferrocyanide promotes lipid oxidation (Hansen et al., 1996; Nguyen et al., 2012).

Overall, the Panel considered that the use of ferrocyanides as food additives is not of genotoxic concern.

3.5.5. Chronic toxicity and carcinogenicity

Neither the SCF (1991) nor JECFA (1970a, 1974a, 1975) have described any data on chronic toxicity and carcinogenicity of sodium, potassium or calcium ferrocyanide.

In a study carried out at BIBRA (British Industrial Biological Research Association) between 1974 and 1976, Wistar rats (48 animals/sex per group, initial body weight 40–60 g) were given 0, 50, 500 or 5,000 mg/kg sodium ferrocyanide decahydrate in the diet (equal to 0, 4.4, 45 and 450.7 mg/kg bw per day for males and 0, 6.2, 62.5 and 630.1 mg/kg bw per day for females) for 2 years (COT, 1994b). The Panel noted that no analyses were carried out to verify the concentrations of ferrocyanide

in the various diets. The Panel further noted that it was a pre-GLP study, but that the BIBRA GLP Unit audited the study to ensure that the results accurately reflects the raw data generated during the study. There are some further inadequacies in the 2-year study as compared by current standards. No clinical biochemistry parameters were measured and several organs were missing for histopathological examination. However, The Panel considered none of the inadequacies large enough to invalidate the study.

Rats were observed on a daily basis for abnormalities of appearance and behaviour or signs of ill-health and weighed regularly throughout the study (first day of treatment; first week; every fortnight during the first year and every month for the remainder of the study). Food and water intake were recorded approximately once a fortnight (first year) and at monthly intervals (second year). Blood was sampled during weeks 14, 26 and 54 from 12 rats/sex from the 0, 500 and 5,000 mg/kg groups and from all surviving rats sacrificed at the end of the study. Blood was analysed for total red blood cell (RBC) and white blood cell (WBC) counts, differential WBC count (0 and 5,000 mg/kg groups only), haemoglobin concentration, packed cell volume (PCV) and reticulocyte count (0 and 5,000 mg/kg groups only). Urine from 12 rats/sex (0 and 5,000 mg/kg groups) and 10 rats/sex (5 and 500 mg/kg groups) was sampled at 2 months intervals until week 104 were urine from all surviving animals was collected. Urine collected over a 6-h period was analysed for volume, specific gravity, pH, glucose, blood, bilirubin, ketones and protein. Urine collected over a 2-h period immediately following an oral water load of 25 mL/kg was analysed for volume, specific gravity and cell content. Urine collected over a 6-h period commencing 18 h after an oral water load of 25 mL/kg was analysed for volume and specific gravity. Animals that died during the study and all animals sacrificed at the end of the study were subjected to a post mortem examination. Major organs were weighed and adrenal, aorta, bladder, brain, caecum, colon, duodenum, eye, Harderian gland, heart, ileum, kidney, liver, lung, lymph node, mammary, muscle, nerve, oesophagus, ovary, pancreas, pituitary, prostate, rectum, salivary, seminal vesicles, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus and vagina were investigated for non-neoplastic and neoplastic findings. The average daily intakes of sodium ferrocyanide were estimated to be equal to 0, 4.4, 45 or 450.7 mg/kg bw per day for males and 0, 6.2, 62.5 or 630.1 mg/kg bw per day for females given 0, 50, 500 or 5,000 mg/kg sodium ferrocyanide in the diet.

Rats (both sexes) from the highest dose group drank more water compared to the controls and the other two dose groups during part of the first 9 months of the study. Except for a higher number of cells excreted in 2-h urine samples from treated animals (all levels of treatment but most frequently in the mid- and high-dose groups and not consistently throughout the study) compared with controls ($p < 0.05$ to $p < 0.001$), no treatment-related adverse effects were observed in the urine analysis. There were higher incidences of pneumonia in male rats from the 5,000 mg/kg group (20/48 animals, $p < 0.01$) and of emphysema in both the 500 and 5,000 mg/kg groups (7/48 animals, $p < 0.01$ and 13/48 animals, $p < 0.001$, respectively) compared to control. No other statistically significant treatment-related effects were reported (COT, 1994b).

In a study performed concurrently with the 2-year study, rats (12 animals/sex per group) were given 0, 50, 500 or 5,000 mg/kg sodium ferrocyanide decahydrate in the diet for 49 weeks (COT, 1994b). Blood was sampled at the end of the study and analysed for total RBC and WBC counts, differential WBC count, haemoglobin concentration, PCV and reticulocyte count. Urine samples were taken at weeks 47–49 from all rats and analysed for bilirubin, glucose, blood, ketones and protein. In addition renal function tests were performed. Full post-mortems and histopathological examination of heart, kidney, liver, lung and spleen was carried out on all rats. There was a statistically significant increase in the mean number of cells excreted per hour in 2-h urine samples of treated animals (both sexes) from the 500 and 5,000 mg/kg groups compared with controls (males: 683, 533, 1,517 and 4,000; females 250, 418, 4,200 and 4,782 in the 0, 50, 500 and 5,000 mg/kg groups, respectively). In addition, the concentrations of urine samples from both sexes in the highest treatment group taken at 6 and 18 h were statistically significantly higher compared to controls.

Overall, no carcinogenic effect was seen in these studies and neither were there any non-neoplastic findings observed considered to be of toxicological relevance. In particular, no treatment-related effects were observed in kidneys of rats given 0, 4.5, 45 or 450 mg sodium ferrocyanide/kg bw per day, for either 49 weeks or 2 years (COT, 1994b). However, in the 2-year study, mid- and high dose animals frequently showed a higher cell excretion rate in 2-h urine samples than did controls. This effect was seen in both males and females, although it was not consistent and inter-animal variation in the parameter was frequently large. An increased cell excretion rate compared to controls was also seen at the low-dose group but only on three occasions. No adverse renal effects were seen at histopathological examination, other than a slight increase in incidence and severity of

glomerulonephrosis in males in the first interim study. Nevertheless, since the kidneys are known to be the target organ for ferrocyanide toxicity, the Panel considered the increased cell excretion rate indicative for occasional, transient kidney toxicity and a NOAEL of 50 mg/kg diet (equal to an intake of 4.4 mg/kg bw per day in male rats and 6.2 mg/kg bw per day in females) was identified.

3.5.6. Reproductive and developmental toxicity

Reproductive toxicity studies

No studies available.

Developmental toxicity studies

Pregnant Crl:CD (SD) BR VAF/Plus strain rats (25 animals/group) were given sodium ferrocyanide once daily by gavage at levels of 0, 100, 500 or 1,000 mg/kg bw/day from gestational day (GD) 6 to 15 (as cited in COT, 1994b). Higher water consumption was noted for all treated groups throughout the study period ($p < 0.05$). There was a marginal higher number of fetuses and litters with larger dilation of the renal pelvis/ureter in the 500 (4/125 fetuses; 4/21 L) and 1,000 mg/kg (5/133 fetuses; 4/23 L) groups compared to the control group. The difference was not statistically significant and in the absence of any associated lesions and the small number of fetuses affected it was concluded by COT (1994b) that a relationship to treatment was considered not to have been proved. The Panel considered the highest dose tested 1,000 mg sodium ferrocyanide/kg bw per day as the NOAEL of this study.

Overall, there were no reproductive studies available and in one prenatal developmental toxicity study in rats (as cited in COT, 1994b) a NOAEL of 1,000 mg sodium ferrocyanide/kg bw per day (the highest dose tested) was identified.

3.5.7. Hypersensitivity, allergenicity and food intolerance

No data were available.

3.6. Discussion

Sodium, potassium and calcium ferrocyanide (E 535, 536 and 538) are anticaking agents authorised as food additives in the EU, previously evaluated by JECFA several times, the latest in 1974 (JECFA, 1975) and the SCF in 1990 (SCF, 1991). The SCF and JECFA established a group ADI of 0–0.025 mg/kg bw per day (calculated as sodium ferrocyanide) for sodium and potassium ferrocyanide, and sodium, potassium and calcium ferrocyanide, respectively.

Specifications for sodium, potassium and calcium ferrocyanide have been defined in the EU in Commission Regulation (EU) No 231/2012 and also by JECFA (2006). The purity is specified to be not less than 99% for sodium, potassium and calcium ferrocyanide.

Potassium ferrocyanide was absorbed to a limited extent from the gastrointestinal tract following oral administration to rats (Gage (1950), cited in JECFA). The majority was excreted unchanged in the faeces (approximately 95% of the dose). Ferrocyanide was detected in liver, spleen, kidney and heart/lungs. Free iron was detected in erythrocytes and free cyanide was detected in urine and expired air. The exposure to free cyanide was estimated to be less than 0.06 mg/kg bw after oral administration to rats of 10 mg ^{59}Fe- and ^{14}C-labelled potassium ferrocyanide per animal (Nielsen et al., 1990a). In humans, absorption was 0.25–0.42% (Nielsen et al., 1990b).

Potassium ferrocyanide is of low acute oral toxicity.

Treatment-related effects were observed in kidneys (higher organ weight, tubular damage and granular and calcified deposits) in rats given 0.5% and 5% sodium ferrocyanide (450 and 4,500 mg/kg bw per day) in the diet for 13 weeks (Oser, 1959; cited in JECFA (1975)). No treatment-related effects were observed in dogs given dietary sodium ferrocyanide up to 25 mg/kg bw per day for 13 weeks (Morgaridge, 1970; cited in JECFA (1975)).

Based on the available data, the Panel considered that the use of ferrocyanides as food additives is not of genotoxic concern.

No carcinogenic effects were observed in rats given 0, 50, 500 or 5,000 mg/kg sodium ferrocyanide in the diet for either 49 weeks or 2 years (COT, 1994b). However, in the 2-year study, mid- and high dose animals frequently showed a higher cell excretion rate in 2-h urine samples than did controls. Since the kidney is known to be the target organ for ferrocyanide toxicity, the Panel considered the increased cell excretion rate indicative for occasional, transient kidney toxicity and identified a NOAEL of 4.4 mg/kg bw per day in male rats and 6.2 mg/kg bw per day in females.

There were no reproductive toxicity studies available and in one prenatal developmental toxicity study in rats (as cited in COT, 1994b) a NOAEL of 1,000 mg sodium ferrocyanide/kg bw per day (the highest dose tested) was identified.

The Panel considered the excretion of a high number of cells in the urine of mid- and high-dose rats in the 2-year study as pivotal effect. Based on the lowest NOAEL for this effect of 4.4 mg sodium ferrocyanide/kg bw per day for male rats, the Panel derived an ADI of 0.044 mg sodium ferrocyanide/kg bw per day. Assuming that the toxicity of this compound is due to the ferrocyanide ion only, the Panel established a group ADI for sodium, potassium and calcium ferrocyanide of 0.03 mg/kg bw per day expressed as ferrocyanide ion. The Panel noted that at this ADI the potential amount of free cyanide released would not be of safety concern.

To assess the dietary exposure to ferrocyanides (E 535–538) from their use as food additives, the exposure was calculated based on (1) MPL in FC 12.1.1 'Salt' set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the mean reported use levels of salt (defined as the *refined exposure assessment scenario*).

The Panel decided to use salt intake data from urinary excretion studies for the assessment of exposure to ferrocyanides (E 535–538) instead of the food consumption data from the EFSA Comprehensive European Food Consumption Database as dietary surveys are commonly not considered as a good source of information in the estimation of salt intake while a more accurate way of estimation of the salt intake is a calculation from the urinary excretion of sodium.

Dietary exposure to ferrocyanides was calculated based on mean and high levels consumption of salts in both the regulatory maximum level and the refined scenario.

In the *MPL scenario*, the exposure to ferrocyanides (E 535–538) from their use as a food additive was up to 0.009 mg/kg bw per day in children and adolescents. In the *refined estimated exposure scenario,* the exposure was up to 0.004 mg/kg bw per day in children and adolescents. Considering that the majority of the use levels in salt reported by Industry were for sodium ferrocyanide (E 535), these exposures would correspond approximately to 0.003 mg ferrocyanide ion/kg bw per day in children and adolescents in the refined exposure scenario.

Information from the Mintel's GNPD showed that from the salt products of subcategory 'Seasonings' only 13% was labelled with ferrocyanides (E 535–538) while in the exposure assessment it was assumed that 100% of the salt consumed contains the additive.

Overall, the Panel considered that the uncertainties identified indicate an overestimation of the exposure to ferrocyanides (E 535–538) as food additives in European countries for both the regulatory maximum level and the refined exposure scenario.

The Panel also noted that the refined exposure estimates are based on information provided on the reported level of use of ferrocyanides (E 535–538). If actual practice changes this refined estimates may no longer be representative and should be updated.

4. Conclusions

Considering that:

- in the refined exposure scenario estimated exposure to ferrocyanides (E 535–538) would correspond approximately to 0.003 mg ferrocyanide ion/kg bw per day in children and adolescents;
- absorption of ferrocyanides from the gastrointestinal tract was low, and there is no accumulation in human;
- ferrocyanides are of low acute toxicity and not mutagenic or carcinogenic;
- reproductive studies were not available, but a NOAEL of 1,000 mg sodium ferrocyanide/kg bw per day (highest dose tested) was identified from a prenatal developmental toxicity study;
- the kidney is the target organ for ferrocyanides toxicity as characterised by the high number of cells excreted in the urine in rats;
- 4.4 mg sodium ferrocyanide/kg bw per day was identified as the NOAEL for this effect in a chronic (2-year) study in rats;
- assuming that the toxicity of this compound is due to the ferrocyanide ion only, the Panel established a ADI for ferrocyanide ion of 0.03 mg/kg bw per day;

- ferrocyanides (E 535–538) are only permitted as food additives in two food categories.

The Panel concluded that ferrocyanides (E 535–538) are of no safety concern in these current authorised use and use levels.

The Panel further concluded that the available data give reason to revise the ADI of 0.025 mg sodium ferrocyanide/kg bw per day (equivalent approximately to 0.02 mg ferrocyanide ion/kg bw per day) based on a subchronic study, to a group ADI for sodium, potassium and calcium ferrocyanide of 0.03 mg/kg bw per day expressed as ferrocyanide ion.

Documentation provided to EFSA

1) Pre-evaluation document on sodium, potassium and calcium ferrocyanide (E 535, 536 and 538). Technical University of Denmark (DTU). Submitted in November 2013.
2) Extensive Literature search covering from January 1990 up to May 2018. Analytical LASER submitted in May 2018.
3) FoodDrinkEurope (FDE), 2017. Data on usage levels of sodium ferrocyanide (E 535) and potassium ferrocyanide (E 536) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 6), Published 23 February 2017. Submitted to EFSA on 29 November 2017.
4) European Potato Processors' Association (EUPPA), 2017. Data on usage levels of sodium ferrocyanide (E 535) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 6), Published 23 February 2017. Submitted to EFSA on 30 November 2017.
5) European Salt Producers' Association (EU_SALT), 2017. Data on usage levels of sodium ferrocyanide (E 535) and potassium ferrocyanide (E 536) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 6), Published 23 February 2017. Submitted to EFSA on 25 November 2017.
6) Ornua, 2017. Data on usage levels of Data on usage levels of sodium ferrocyanide (E 535) and potassium ferrocyanide (E 536) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 6), Published 23 February 2017. Submitted to EFSA on 29 November 2017.
7) Intersnack, 2017. Data on usage levels of potassium ferrocyanide (E 536) and calcium ferrocyanide (E 538) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 6), Published 23 February 2017. Submitted to EFSA on 29 November 2017.

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Abbreviations

ADI	acceptable daily intake
ANS	EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food
BIBRA	British Industrial Biological Research Association
bw	body weight
CAS	Chemical Abstracts Service
CONTAM	EFSA Panel on Contaminants in Food Chain
COT	UK Committees on the Toxicity of Chemicals in Food, Consumer Products and the Environment
CV	crystal violet
EINECS	European Inventory of Existing Chemical Substances
EUPPA	European Potato Processors' Association
EU_SALT	European Salt Producers' Association
EV	ethyl violet
FAO	Food and Agriculture Organization of the United Nations

FCs	food categories
FCS	food categorisation system
FDE	FoodDrinkEurope
FFA	free fatty acids
FI	flow injection
GD	gestational day
GNPD	Global New Products Database
HPLC	high-performance liquid chromatography
IG	iodine green
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
MeG	methyl green
MPL	maximum permitted level
MS	mass spectrometry
MV	methyl violet
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCV	packed cell volume
RBC	red blood cell
RRS	resonance Rayleigh scattering
RSD	relative standard deviation
SCAN	Scientific Committee for Animal Nutrition
SCF	Scientific Committee on Food
TBARS	thiobarbituric acid-reactive substances
TemaNord	is a publishing series for results of the often research-based work that working groups or projects under Nordic Council of Ministers have put in motion
WBC	white blood cell
WBR	whole body retention
WG	Working Group
WHO	World Health Organization

Appendix A – Summary of reported use levels (mg/kg or mg/L as appropriate) of E 535–538 ferrocyanides provided by industry

Appendix A can be found in the online version of this output ('Supporting information' section).

Appendix B – Number and percentage of food products labelled with E 535–538 ferrocyanides out of the total number of food products present in the Mintel GNPD per food subcategory between 2013 and April 2018

Appendix B can be found in the online version of this output ('Supporting information' section).

Appendix C – Number Available data on daily sodium urinary excretion in children and adults in European countries and their estimated salt intake used for the exposure assessments

Appendix C can be found in the online version of this output ('Supporting information' section).

Appendix D – Summary of total estimated exposure of ferrocyanides (E 535–538) from their use as food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and high exposure (mg/kg bw per day)

Appendix D can be found in the online version of this output ('Supporting information' section).