Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

tetrairon tris(pyrophosphate); ferric pyrophosphate

EC Number: 233-190-0
CAS Number: 10058-44-3

CLH-O-0000007280-81-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 March 2023

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification: tetrairon tris(pyrophosphate); ferric pyrophosphate

EC Number: **233-190-0**

CAS Number: **10058-44-3**

Index Number: **-**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TETRAIRON TRIS(PYROPHOSPHATE); FERRIC PYROPHOSPHATE

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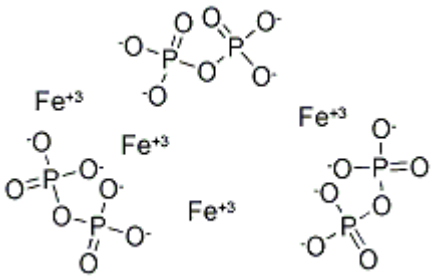
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	tetrairon tris(pyrophosphate)
Other names (usual name, trade name, abbreviation)	iron (III) pyrophosphate, diphosphoric acid iron (III) salt
Common name (if available and appropriate)	ferric pyrophosphate
EC number (if available and appropriate)	233-190-0
EC name (if available and appropriate)	tetrairon tris(pyrophosphate)
CAS number (if available)	10058-44-3
Other identity code (if available)	-
Molecular formula	$Fe_4(P_2O_7)_3$
Structural formula	
SMILES notation (if available)	[[O-]P(=O)([O-])OP(=O)([O-])[O-].[O-]P(=O)([O-])OP(=O)([O-])[O-].[O-]P(=O)([O-])OP(=O)([O-])[O-].[Fe+3].[Fe+3].[Fe+3].[Fe+3]
Molecular weight or molecular weight range	745.21g/mol (anhydrous)
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 80.2% (w/w) pure anhydrous active substance in technical active substance

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
tetrairon	≥80.2% (w/w)	Not applicable	Not Classified

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
tris(pyrophosphate) EC no.: 233-190-0			

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives					

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Not applicable - The composition of the tested substance is the same as the substance covered by this CLH proposal with purity ≥ 80.2%				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	-	tetrairon tris(pyrophosphate); ferric pyrophosphate	233-190-0	10058-44-3	Eye Irrit.2	H319	GHS07 Wng	H319	-	-	-
Resulting Annex VI entry if agreed by RAC and COM		tetrairon tris(pyrophosphate); ferric pyrophosphate	233-190-0	10058-44-3	Eye Irrit.2	H319	GHS07 Wng	H319			

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	hazard class not applicable	No
Flammable solids	data conclusive but not sufficient for classification	Yes
Self-reactive substances	data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	hazard class not applicable	No
Pyrophoric solids	data conclusive but not sufficient for classification	Yes
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	data conclusive but not sufficient for classification	Yes
Oxidising liquids	hazard class not applicable	No
Oxidising solids	data conclusive but not sufficient for classification	Yes
Organic peroxides	hazard class not applicable	Yes
Corrosive to metals	data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	data lacking	Yes
Acute toxicity via inhalation route	data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	harmonised classification proposed	Yes
Respiratory sensitisation	data conclusive but not sufficient for classification	Yes
Skin sensitisation	data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data conclusive but not sufficient for classification	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification	Yes
Aspiration hazard	data conclusive but not sufficient for	Yes

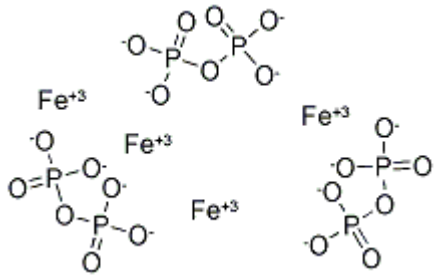
Hazard class	Reason for no classification	Within the scope of public consultation
	classification	
Hazardous to the aquatic environment	data conclusive but not sufficient for classification	Yes
Hazardous to the ozone layer	data conclusive but not sufficient for classification	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Ferric pyrophosphate is not listed in Annex VI of Regulation (EC) No 1272/2008. Ferric pyrophosphate was not classified according to Directive 67/548/EEC.

RAC general comment

About this substance



$Fe_4(P_2O_7)_3$; iron (III) pyrophosphate

Tetrairon tris (pyrophosphate); ferric pyrophosphate is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area at ≥ 100 to < 1000 tonnes per annum.

This substance is used by consumers and professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing of several products such as coating products, plasters, inks, pest control products, food products and food supplements. It is also a pesticidal active substance under (EC) 1107/2009.

Dossier Submitter's classification proposal

According to Annex VI, part 2 of the CLP Regulation, the information provided in the REACH Registration dossier was included in the CLH report and considered in this opinion.

This RAC opinion is mainly based on the available data from the CLH report which were included in the Renewal Assessment Report developed in accordance with the Commission Regulation (EC) No. 844/2012.

Ferric pyrophosphate is characterised by a low bioavailability following oral administration. Due to the poor solubility in water and lipids the absorption in the body is low, and it does not accumulate in the organism. A low level of iron excretion is observed under normal physiological conditions.

Given the prevalence in nature of iron and phosphorus, and the potential absorption of ferric pyrophosphate from water, exposure to this substance will not increase significantly as a result of its use in plant protection products. Exposure related to absorption of pyrophosphate in other ways is not expected as the substance is non-volatile and the product has a form of non-dusty granules.

Data on exposure cited from acknowledged scientific sources combined with low toxicity evidenced in the studies presented indicate that further toxicological studies are not yet necessary.

Regarding the completeness of the data in the CLH report, RAC concludes that the applicant submitted a limited data package with the ferric pyrophosphate including acute oral and inhalation toxicity, eye and skin irritation studies, a genotoxicity test battery and short-term oral toxicity studies in rats.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

This CLH Report is mainly based on the available data from the Draft Assessment Report for Ferric pyrophosphate available via link: http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?0-1.ILinkListener-outputForm-outputDocumentsContainer-documents-2-fileNameLnk developed in accordance with Regulation 1107/2009 and the Regulation (EC) No. 844/2012 by the Polish CA.

5 IDENTIFIED USES

Products containing ferric pyrophosphate is to be used in agriculture and horticulture for control of harmful slug and snail species in all edible and inedible plants grown in the filed conditions and under protection.

6 DATA SOURCES

This CLH Report is mainly based on the available data from the Draft Assessment Report for Ferric pyrophosphate available via link: http://registerofquestions.efsa.europa.eu/roqFrontend/wicket/page?12 developed in accordance with Regulation 1107/2009 and the Regulation (EC) No. 844/2012 by the Polish CA.

Because REACH registration dossier for tetrairon tris(pyrophosphate) (EC 233-190-0) is available https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/12264, according to Annex VI, part 2 of the CLP regulation the information provided in registration dossier concerning the hazard classes included in this CLH report are evaluated and summary of the assessment are included in this report.

Systematic literature search and relevant publications found.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid, fine powder, very light shade of beige, a delicate, slightly noticeable characteristic odour		
Melting/freezing point	Melting point > 360°C	M. Włodarczak 2015	Measured (EU A.1.)

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
	Melting point > 450°C at 101.3 kPa	J. Walker 2009 (REACH registration dossier)	Measured (EU A.1.)
Boiling point	Waived	-	The sample melts above 300°C, therefore the study was not conducted.
Relative density	2,524 g /cm^3	M. Włodarczak 2012;	Measured (EU A.3).
	2,967 g /cm^3	REACH registration dossier	OECD 109
Vapour pressure	Waived	-	The sample melts above 300°C, therefore the study was not conducted.
Surface tension	Waived	-	EC method A.5 states that a water solubility of ≥ 1mg/L is needed. Ferric pyrophosphate solubility is lower, therefore this study was not conducted.
Water solubility	Temperature 20±0.5°C pH 4 (24h – 140.3 µg/l; 48h – 164.8 µg/l; 72h – 141.7 µg/l) pH 7 (24h – 41.2 µg/l; 48h – 41.6 µg/l; 72h – 39.0 µg/l) pH 9 (24h – 135.9 µg/l; 48h – 113.1 µg/l; 72h – 112.3 µg/l)	M. Włodarczak 2015	Measured. (OECD 105)
	367µg/l at 20.0 ± 0.5°C pH 4 -72h – 297 µg/l at 20.0 ± 0.5°C pH 9 -72h – 252x 10^{-3} µg/l at 20.0 ± 0.5°C	REACH registration dossier	Measured (EU A.6)
Partition coefficient n-octanol/water	Waived	-	Not required for inorganic substance. Ferric pyrophosphate is practically insoluble in water.
Flash point	Waived	-	Not required for inorganic substance. Ferric pyrophosphate is solid. Therefore, it is not possible to determine flash point.
Henry's law constant	Waived	-	Pursuant to Column II of Annex VII to Commission Regulation (EC) No 1907/2006 the study does not need to be conducted if the melting point is above 300 °C. On this basis testing is not required because melting point for ferric pyrophosphate is above 360°C

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Flammability	Not highly flammable	M. Włodarczak 2015	Measured (EU A.10.). Purity: 101,73% (as hydrate)
Explosive properties	No explosive properties	-	A theoretical estimation based on structure.
Self-ignition temperature	Not self-ignitable	M. Włodarczak 2015	Measured (EU A.16.). Purity: 101,73% (as hydrate)
Oxidising properties	No oxidising properties	-	A theoretical estimation based on chemical structure.
Granulometry	Data lacking	-	-
Stability in organic solvents and identity of relevant degradation products	Waived	-	Not required for inorganic compounds.
Dissociation constant	pKa1 = 0.1 (25ºC) pKa2 = 2.31 (25ºC) pKa3 = 6.69 (25ºC) pKa4 = 9.42 (25ºC)	REACH registration dossier	No experimental determination of the dissociation constants in water was performed for the test materials as it was anticipated that on performance of the test procedures, as detailed in Method 112 of the OECD Guidelines for Testing of Chemicals, 12 May 1981, that the resulting dissociation constants determined would be that of the parent anions only, for which literature values are available. For example, it is anticipated that different types of orthophosphate will demonstrate significantly different pH values in water due to increasing numbers of protons being substituted with the particular cation on titration with acid or base as appropriate, the actual dissociation constants determined for each compound would be common, i.e. that of the triprotic acid anion. Titrations would also be expected to be similar irrespective of the counter ion. Read-across is justified on the basis that pyrophosphoric acid is the parent acid for all inorganic pyrophosphates.

Property	Value	Reference	Comment (e.g. measured or estimated)
Viscosity	Waived	-	Not applicable for solid substance. Ferric pyrophosphate is a powder.

8 EVALUATION OF PHYSICAL HAZARDS

Explosives

Short summary and overall relevance of the information provided on explosive properties

Ferric pyrophosphate has no chemical group associated with explosive properties present in the molecule.

Comparison with the CLP criteria

According to Part 2, 2.1.4.3 a) of Annex I of CLP Regulation, ferric pyrophosphate shall not be classified as explosive considering that are no chemical groups associated with explosive properties (given in Table A6.1 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria) in the molecule.

Therefore no classification according to the CLP criteria for explosive properties is warranted.

Conclusion on classification and labelling for explosive properties

No classification is proposed for ferric pyrophosphate regarding explosives hazards according to CLP criteria.

Flammable gases (including chemically unstable gases)

Not applicable - substance is not in the applicable physical state for the hazard class in question.

Oxidising gases

Not applicable - substance is not in the applicable physical state for the hazard class in question.

Gases under pressure

Not applicable - substance is not in the applicable physical state for the hazard class in question.

Flammable liquids

Not applicable - substance is not in the applicable physical state for the hazard class in question.

Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EU A.10.	Not highly flammable.	Purity: 101.73% (as hydrate)	M. Włodarczak 2015

Short summary and overall relevance of the provided information on flammable solids

No ignition of test item strip was observed over 2 minutes of constant hot flame application. Because no ignition was observed during preliminary test, burning rate test was not conducted.

Comparison with the CLP criteria

According to Part 2, 2.7.2.1 of Annex I of CLP Regulation, ferric pyrophosphate shall not be classified as flammable considering that no ignition of test item strip was observed over 2 minutes of constant hot flame application.

The method used for classification purposes according to CLP criteria is the UN Test N.1 described in the UN RTDG, Manual of Tests and Criteria (7th revision). However, as reflected in the ECHA Guidance on Information Requirements and Chemical Safety Assessment (R.7.1.10.3), if the result of an A.10 method indicates that classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary.

Ferric pyrophosphate was classified as ‘not highly flammable’ in the EC Method A.10. Therefore, no classification according to the CLP criteria for flammability is warranted.

Conclusion on classification and labelling for flammable solids

No classification is proposed for ferric pyrophosphate regarding flammable solids hazards according to CLP criteria.

Self-reactive substances

The study does not need to be conducted because there are no chemical groups present in the molecule which are associated with explosive or self-reactive properties and hence, the classification procedure does not need to be applied.

Ferric pyrophosphate is not thermally unstable solid substance liable to undergo a strongly exothermic decomposition even without participation of oxygen (air).

Therefore, no classification according to the CLP criteria for self-reactive substances is warranted.

Pyrophoric liquids

Not applicable - substance is not in the applicable physical state for the hazard class in question.

Pyrophoric solids

The study does not need to be conducted because ferric pyrophosphate is known to be stable into contact with air at room temperature for prolonged periods of time and hence, the classification procedure does not need to be applied.

No classification according to the CLP criteria for pyrophoric solids is warranted.

Self-heating substances

Table 10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EU A.16.	Not self-ignitable.	Purity: 101.73% (as hydrate)	M. Włodarczak 2015

Short summary and overall relevance of the provided information on self-heating substances

There are not noticeable the exothermic or endodermic changes of the sample between the temperature of oven 20-400°C connected with self-ignition of the substance or phase changing and melting of the test substance. The test item does not ignite until the temperature of 400°C.

Comparison with the CLP criteria

According to Part 2, 2.11.2.1 of Annex I of CLP Regulation, ferric pyrophosphate shall not be classified as self-heating considering that no self-ignition of test item was observed until the temperature of 400°C.

Therefore no classification according to the CLP criteria for self-heating is warranted.

Conclusion on classification and labelling for self-heating substances

No classification is proposed for ferric pyrophosphate regarding self-heating substances hazards according to CLP criteria.

Substances which in contact with water emit flammable gases

The study does not need to be conducted because ferric pyrophosphate by interaction with water is not liable to become spontaneously flammable or to give off flammable gases in dangerous quantities. The experience in production or handling shows that the substance does not react with water.

No classification according to the CLP criteria for substances which in contact with water emit flammable gases is warranted.

Oxidising liquids

Not applicable - substance is not in the applicable physical state for the hazard class in question.

Oxidising solids

According to definition in Annex I:

2.14.1. O*xidising solid means a solid substance or mixture which, while in itself is not necessarily combustible, may, generally by yielding oxygen, cause, or contribute to, the combustion of other material.*

Ferric pyrophosphate contains oxygen therefore the classification as oxidising solid should be considered.

Pyrophosphate is non-oxidizing ion (originate from non-oxidizing acid) and disconnection of the oxygen from the pyrophosphate group is very difficult due to phosphorus high affinity to oxygen. Phosphorus is one of the strongest reducers (phosphorus seeks to the highest oxidation state, where it is stable). Phosphorous when bound to oxygen is in a stable state and reducing it to elemental P is very difficult, requiring extreme conditions and very strong reducing agents, extreme conditions and very strong reducing agents.

Based on above justification ferric pyrophosphate is not capable yielding oxygen therefore no classification according to the CLP criteria for oxidizing solids is warranted.

Organic peroxides

The study does not need to be conducted because the substance does not fall under the definition of organic peroxides.

Corrosive to metals

The study does not need to be conducted because ferric pyrophosphate is stable substance not reacting with metals, by chemical action will not materially damage, or even destroy, metals. From the structural formula and composition of the substance it can be concluded that ferric pyrophosphate does not have to be classified as corrosive to metals.

No classification is warranted for ferric pyrophosphate regarding all physico-chemical hazardous properties based on Table 8 above.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Ferric pyrophosphate is a solid inorganic substance, therefore only hazard classes relevant for solids were open for consultation. The DS proposed no classification for all the relevant hazard classes based on the chemical structure (explosives, self-reactive substances, oxidising solids, organic peroxides, and corrosive to metals), on experience (pyrophoric solids, and substances which in contact with water emit flammable gases), or based on study results (flammable solids, and self-heating substances).

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that the screening procedure for explosives or self-reactive substances based on the chemical structure is applicable for organic substances only, while ferric pyrophosphate is an inorganic compound. RAC concludes on no classification for explosives and self-reactive substances due to lack of data.

For oxidising solids, the screening procedure is applicable to inorganic substance which do not contain oxygen or halogen atoms. Ferric pyrophosphate does contain oxygen atoms, thus the screening procedure is not applicable and the UN RTG O.1 test should have been conducted. RAC concludes on no classification for oxidising solids due to lack of data.

RAC notes that based on the structure the hazard class "organic peroxide" is not relevant for an inorganic substance.

RAC agrees with the DS on no classification based on experience for pyrophoric solids and substances which in contact with water emit flammable gases.

RAC agrees with the DS on no classification as flammable solids based on a negative EU A.10 test (not highly flammable, see Table 9 of the CLH report).

RAC agrees with the DS on no classification for self-heating substances based on a negative EU A.16 test method.

RAC agrees with the DS, that ferric pyrophosphate should not be classified as corrosive to metals.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 11**: Summary table of toxicokinetic studies**

Method	Results	Remarks	Reference
No study submitted - Justification for non-submission accepted for the plant protection product procedure.			

Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Iron absorption plays the major role in maintaining homeostasis in the human body. Only a fraction of daily ingested iron is absorbed. Iron absorption takes place in the entire intestine but mainly in the duodenum. Considerably lower amounts are absorbed in the stomach. Iron absorption depends on several factors such as its content in food, stores in the organism and its form in food. Iron is absorbed in the form of divalent cations, whereas ferric ion (Fe^{3+}) is released from food as a result of digestion by gastric acid in the stomach, next it is reduced to ferrous ion (Fe^{2+}) and only then it is absorbed. Ferrous ion (Fe^{2+}) comprises about 10% of the daily iron supply in food and it is absorbed in about 20%. Its absorption is decreased by calcium present in food. On the other hand, ferric ion (Fe^{3+}) comprises 80% of the daily iron supply in food and its absorption is low - from 1 to 5% - and depends on other components of food. Iron transport through membranes requires energy and is supported by carriers, thus the process might become saturated and decrease the speed of iron absorption. Ferrous ion is absorbed by the mucosa of the gastrointestinal tract and then converted to the ferric state. In intestinal epithelial cells, it binds to apoferritin, forming ferritin. Transported from the epithelial cells to blood, iron binds to transferrin - protein which transports iron to the bone marrow, where it is used in erythropoiesis.

Organisms are protected from the toxic effect of iron mainly by the liver (also the spleen and bone marrow to a lesser extent), where it is stored in the form of soluble complex of ferritin and hemosiderin (its insoluble derivative). The excessive amounts of the stored iron can be released any time from this buffer pool. About 25% of the total amount of iron is stored in the liver (about 2/3 as ferritin and 1/3 as hemosiderin).

Cells absorb iron through receptors binding transferrin to Fe^{3+}, which are transmembrane proteins consisting of two glycoprotein monomers connected with a sulfur bridge. On the inner side of the cell membrane, there are fatty acids linked with the proteins by covalent bonds. Receptor-transferrin-iron complex is absorbed via endocytosis, forming vacuoles in the cytoplasm. Acidic environment of vacuoles causes transferrin to release iron, then the receptor-transferrin complex is transferred back to the cell surface, ready for another round of iron uptake.

The extent of iron absorption demonstrates intra- and inter-subject variability, which is mainly influenced by dietary factors and characteristics of the organism itself (age, sex, health condition etc.) The ingested substances might modify the level of absorption e.g. by chelating and/or change in iron oxidation level, effect on the mucosa and function of intestines, or the competitive mechanism of other minerals in protein transport. Inductors and inhibitors of iron absorption are provided with food. The former include e.g. meat and vitamin C, the latter - calcium, polyphenols, phosphates, carbonates and soy proteins. Induction and inhibition of absorption by these dietary components is closely related to redox processes and formation of soluble monomers or insoluble polymers. Anions which form relevant salts with Fe cations also determine the size of the absorbed dose because they differ in the level of solubility (ferric pyrophosphate is an insoluble compound, whereas ferric sulphate is a highly soluble salt). If iron is provided in an assimilable

form, the size of the dose provided and current demand in the organism will constitute factors influencing the absorption level.

Human organism has a very limited ability to remove excess iron, thus protection from overload with this mineral consists in limiting its absorption. The only natural ways of daily iron loss are epidermis exfoliation and sweating (0.2-0.3 mg/day), excretion in urine (<0.1 mg/day), gastrointestinal secretion and deposition in hair. The total daily loss of iron in healthy men is about 1 mg. In women, this amount is somewhat higher due to menstruation, pregnancy and lactation.

Due to poor iron absorption from food and food processing, developed countries for years have been fortifying food products with sources of iron. In Great Britain, the obligation to add iron compounds to flour has existed since 1953 - flour needs to contain no less than 1.65 mg of iron/100 g. The European law, on the other hand, states that modified milk for infants based on cow's milk should contain 0.07-0.3 mg of iron/100 kcal. Certain food products such as cereal bars or breakfast cereals are fortified by manufacturers even though there is no binding guideline (content ranges between 70 and 120 mg/kg). What is popular is prophylactic supplementation, where dosing amounts to 7-50 mg/day.

According to WHO/FAO recommendations, the substance that should be used for iron supplementation in food in the first place is ferric sulphate, in the last place - ferric pyrophosphate. Even though ferric pyrophosphate is poorly absorbed, it is used in diet fortification as a compound that causes no organoleptic changes in food. Ferric pyrophosphate has been approved as a safe and effective source of iron added to food, even in infants. In accordance with Regulation (EU) No. 609/2013 of the European Parliament and of the Council of 12 June 2013, it was approved for use in baby food for infants and young children, processed cereal-based foods and food for children, food for special medical purposes, and total diet replacement. Also the Food and Drug Administration (FDA) positively assessed ferric pyrophosphate, placing it on the list of substances generally recognized as safe (GRAS).

Ferric pyrophosphate is virtually insoluble in water, which makes it hard to assimilate. Research showed that average absorption of iron from food fortified with pyrophosphate was only 2%.

Ferric pyrophosphate is characterised by the low bioavailability following oral administration. Due to the poor solubility in water and lipids the absorption in the body is low. Ferric pyrophosphate does not accumulate in the organism and the main resources of iron are stored in liver. A low level of iron excretion is observed under normal physiological conditions.

Based on the properties of ferric pyrophosphate, it is considered acceptable that no studies on metabolism and toxicokinetics were submitted for the plant protection product procedure.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

Acute toxicity - oral route

Table 12: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 420 with exception of following deviation: the relative air humidity during the experiment was lower than 30% a few times. These changes did not influence the results of	Rat, Wistar, 6 F	Ferric pyrophosphate Batch 120327086	300 mg/kg b.w. 14-days exposure, 2000 mg/kg b.w 14-days exposure	LD_{50}> 2000 mg/kg bw	Anonymous 1, 2013, Report No. PO-2/13

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
the experiment and the study is considered suitable for evaluation. GLP					
OECD 420 GLP	Rat, Wistar, 5 F	Ferric pyrophosphate (CAS no.: 233-190-0)	2000 mg/kg bw 14-days exposure	LD_{50}> 2000 mg/kg bw	Anonymous 2, 2012a, Report No 41201540

Short summary and overall relevance of the provided information on acute oral toxicity

In both OECD 420 studies an oral limit test was performed in 5 fasted female rats with a single dose of 2000 mg/kg bw of ferric pyrophosphate. No mortalities and no clinical signs were observed in treated animals. All animals gained body weight over the study period. No pathological changes were observed at necropsy. The oral LD_{50} value of ferric pyrophosphate in female rats was established as exceeding 2000 mg/kg bw.

Comparison with the CLP criteria

A LD_{50} > 2000 mg/kg bw was obtained which stands above the highest cut-off value of 2000 mg/kg bw/day from category 4 of the CLP. Therefore Ferric Pyrophosphate doesn't warrant classification for this toxicity hazard.

Conclusion on classification and labelling for acute oral toxicity

No classification in regard to acute oral toxicity is required for ferric pyrophosphate according to criteria of the Regulation 1272/2008.

Acute toxicity - dermal route

Due to the fact that the substance's acute oral toxicity, LD_{50}, is higher than 2000 mg/kg bw according to Commission Regulation (EU) No. 283/2013 a study of acute dermal toxicity is not necessary. The justification for waiving the acute dermal toxicity study of ferric pyrophosphate is scientifically justified and acceptable for plant protection product procedure.

Conclusion on classification and labelling for acute dermal toxicity

No harmonised classiffication is proposed for acute dermal toxicity due to lack of data.

Acute toxicity - inhalation route

Table 13: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403 GLP	Rat, Wistar, 3 M + 3 F	Ferric pyrophosphate Batch 120327086	2.69 mg/L air, 4-hr exposure	LC_{50} >2.69 mg/L air (maximum attainable concentration)	Anonymous 3, 2013, Report No. 4150

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 436 GLP	Rat, Wistar, 3 M + 3 F	Ferric pyrophosphate (CAS no.: 233-190-0)	5.19 mg/L air, 4-hr exposure	LC_{50} >5.19 mg/L air (maximum attainable concentration)	Anonymous 4, 2012, Report No. 41201541

Short summary and overall relevance of the provided information on acute inhalation toxicity

Acute inhalation (4h, nose-only) toxicity testing (Anonymous 3) of ferric pyrophosphate was performed in three male and three female rats. No mortality was recorded throughout the study to the technically highest attainable concentration of 2.69 mg test substance/l air. All exposed animals appeared normal throughout the experimental period. All animals had gained body weight over the study period. No abnormalities were detected in any of the animals on necropsy at the end of observation period.
The acute inhalation LC_{50} (4h) of ferric pyrophosphate for male and female rats was > 2.69 mg/L air (highest technically attainable concentration).
In the second study (Anonymous 4) no deaths occurred in a group of six rats exposed to a mean achieved atmosphere concentration of 5.19 mg/l air for four hours. Thus LC_{50} (4 h) of ferric pyrophosphate for male and female rats was > 5.19 mg/L air.

Comparison with the CLP criteria

The acute inhalation LC_{50} of a dust aerosol of ferric pyrophosphate are greater than 2.69 mg/L and 5.19 mg/L air which is maximum attainable concentration This value is below the upper limit for classification in the least stringent category (i.e. inhalation (dust/mist) LC_{50} > 1 but ≤ 5 mg/l) thus, strictly, it is not possible to exclude that the substance would meet criteria for classification in category 4. Taking into account that 2.69 mg/L was the highest technically attainable concentration, all animals gained weight and no deaths occurred during the study, no classification is proposed with respect to acute toxicity via inhalation. No classification can be confirmed by the LC_{50} > 5.19 mg/L air obtained in the second study Anonymous 4.

Conclusion on classification and labelling for acute inhalation toxicity

No classification in regard to acute inhalation toxicity is required for ferric pyrophosphate according to criteria of the Regulation 1272/2008.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity - oral route

Two studies performed according with GLP and guideline compliant (OECD TG 420) with ferric pyrophosphate are available, both on rats (see Table 12 of the CLH report). The acute oral LD_{50} was > 2000 mg/kg bw in both of them.
The DS proposed no classification for acute toxicity based on the LD_{50} values > 2000 mg/kg bw.

Acute toxicity - dermal route

Because the acute oral LD_{50} value for ferric pyrophosphate is above 2000 mg/kg bw, the acute dermal toxicity study is not necessary according to Commission Regulation (EU) No. 283/2013. Consequently, there is not acute dermal study in the CLH report, and the DS proposed no classification for acute dermal toxicity for ferric pyrophosphate due to lack of data.

Acute toxicity - inhalation route

Two studies on acute inhalation toxicity (4h, nose-only) using ferric pyrophosphate were performed according with GLP on Wistar rats. The LC_{50} was above the stated maximum attainable concentrations of 2.69 mg/L for the first study (OECD TG 403), and of 5.19 mg/L for the second one (OECD TG 436) (see Table 13 of the CLP report).
The DS proposed no classification for ferric pyrophosphate with respect the trigger for acute toxicity – inhalation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Acute oral toxicity

Study 1, Anonymous 1, 2013

The test substance was ferric pyrophosphate (purity 101.73%), and was administered to 6 female Wistar rats (age: 9-11 weeks), at 300 and 2000 mg/kg bw during 14-days of exposure. The study followed the OECD TG 420 with a deviation regarding the relative air humidity which was lower than 30% a few times, but this had no influence on the results. Air humidity was recorded to vary between 5 to 60%, due to the regulation of the air-conditioning device at that time. Water was available for the animals all the time. The test item in the form of a suspension in 0.5% carboxymethylcellulose in a volume of 0.5 mL/100 g bw was administered using a metal stomach tube. No mortalities or clinical signs were observed in the study, and the gross examinations of the animals did not reveal any pathological changes. The acute oral LD_{50} was found to be > 2000 mg/kg bw in all 5 Wistar rats female.

Study 2, Anonymous 2, 2012a

A single dose of 2000 mg/kg bw Ferric pyrophosphate was administered by the oral route to 5 female Wistar rats . The test item in the form of a suspension in 0.5% carboxymethylcellulose in a volume of 0.5 mL/100 g bw was administered using a metal stomach tube. No mortalities or clinical signs were noted in exposed animals; the animals increased their body weight during the observation period and no pathological changes were observed at necropsy. The acute oral LD_{50} was found to be > 2000 mg/kg bw.

In both studies, the LD_{50} was above the cut-off value of 2000 mg/kg bw for classification in Category 4. RAC agrees with the DS proposal of **no classification for acute oral toxicity**.

Acute toxicity - dermal route

No acute dermal study is included in the CLH report, therefore RAC considers that **no**

classification for acute dermal toxicity is warranted due to lack of data.

Acute toxicity - inhalation route

Study 1, Anonymous 3, 2013
Ferric pyrophosphate, purity 101.73%, was administered by inhalation for 4 hours, nose-only, to 3 male and 3 female Wistar rats. The maximum attainable concentration was of 2.69 mg /L. No mortalities were observed during the study, and no abnormalities were detected in any of the animals on necropsy at the end of observation period. The study is reliable, and the LC_{50} was estimated to be > 2.69 mg/L air.

Study 2, Anonymous 4, 2012
Ferric pyrophosphate, purity 101.73%, was administrated by inhalation to 3 male and 3 female Wistar rats at a concentration of 5.19 mg/L, for 4 hours, nose-only. No mortalities were recorded during the study, and no abnormalities were detected in any of the animals on necropsy at the end of observation period. The study is reliable, and the LC_{50} was estimated to be > 5.19 mg/L air.

The acute inhalation LC_{50} of a dust aerosol of ferric pyrophosphate was higher than both the tested concentrations of 2.69 and 5.19 mg/L.
In the CLP Regulation, the cut-off criteria for classification in Category 4 for acute inhalation (dust/mist) is $1 < LC_{50} \leq 5$ mg/L). In the second study (Anonymous 4, 2012), the tested concentration of 5.19 mg/L is above the classification criteria and no mortalities were observed. In the other study (Anonymous 3, 2013), the LC_{50} was above the highest technically attainable concentration of 2.69 mg/L; also in this study no mortalities (or significant changes in the animals) were observed. RAC agrees with the DS proposal of **no classification for acute inhalation toxicity**.

Conclusion on classification and labelling for acute toxicity

RAC considers that no classification is warranted for acute toxicity via all routes of exposure.

Skin corrosion/irritation

Table 14: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Method B.4, Council Regulation (EC) No.440/2008 GLP	Rabbit, New Zealand (albino), 3 F	Ferric Pyrophosphate Batch 120327086	0.5 g, 4 hours	-In initial test three patches were applied sequentially to one animal (rabbit no. 13). Because no corrosive or severe irritant effect was observed even after 4-hour exposure, the response was further observed in regular time intervals at 1, 24, 48 and 72 hours after 4-hour exposure. Because during the initial test no corrosive or severe irritating effect was observed, two additional animals (rabbits no. 14 and 15) were used to confirm the negative response. No skin reaction was observed during any of observation periods. There was no evidence of a corrosive effect on	Anonymous 5, 2013, Report No. 13-154

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance,</th><th>Dose levels duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td><td>the skin. No symptoms of systemic toxicity were observed in the animals during the test period and no mortality occurred. No skin reaction was observed in all rabbits. At 1, 24, 48 and 72 hours after exposure no signs of erythema and oedema were recorded.</td><td></td></tr>
<tr><td>Reconstituted human epidermis model (reconstituted human epidermis model)
OECD 439
GLP</td><td>-</td><td>tetrairon tris(pyrophosphate)
Batch number: 2-47501-56</td><td>- The test Material was applied neat.
- Amount(s) applied (volume or weight with unit):
Approximately 10 mg of the test item was applied to the epidermis surface. The epidermis surface had previously been moistened with 5 µl of sterile distilled water to improve contact between the solid test item and the epidermis.
Duration of treatment / exposure 15 minute exposure & 42 hour post-exposure incubation</td><td>not irritating
Viability of cells: 110.7 of max. 100
Mean OD540 Values and Percentage Viabilities for the Negative Control Material, Positive Control Material and Test Material:
<table>
<tr><th>Material</th><th>OD_{540} of tissues</th><th>Mean OD_{540} of triplicate tissues</th><th>±SD of OD_{540}</th><th>Relative individual tissue viability (%)</th><th>Relative mean viability (%)</th><th>± SD of Relative mean viability (%)</th></tr>
<tr><td rowspan="3">Negative Control Material</td><td>0.666</td><td rowspan="3">0.659</td><td rowspan="3">0.041</td><td>101.1</td><td rowspan="3">100*</td><td rowspan="3">6.2</td></tr>
<tr><td>0.696</td><td>105.6</td></tr>
<tr><td>0.615</td><td>93.3</td></tr>
<tr><td rowspan="3">Positive Control Material</td><td>0.059</td><td rowspan="3">0.056</td><td rowspan="3">0.006</td><td>9.0</td><td rowspan="3">8.6</td><td rowspan="3">1.0</td></tr>
<tr><td>0.061</td><td>9.3</td></tr>
<tr><td>0.049</td><td>7.4</td></tr>
<tr><td rowspan="3">Test Material</td><td>0.769</td><td rowspan="3">0.730</td><td rowspan="3">0.069</td><td>116.7</td><td rowspan="3">110.7</td><td rowspan="3">10.5</td></tr>
<tr><td>0.650</td><td>98.6</td></tr>
<tr><td>0.770</td><td>116.8</td></tr>
</table>
SD= Standard deviation
*= The mean viability of the negative control tissues is set at 100%</td><td>Anonymous 6, 2012a, Report no. 41201542</td></tr>
<tr><td>Reconstituted human epidermis model (reconstituted human epidermis model)
OECD 431
GLP</td><td>-</td><td>tetrairon tris(pyrophosphate)
Batch number: 2-47501-56</td><td>The test item was applied neat.
20 mg of the solid test item was applied topically to the corresponding tissues ensuring uniform coverage of the tissues.</td><td>not irritating
Viability of cells: 110.7 of max. 100
The relative mean viability of the test material treated tissues was as follows:
240 minutes exposure : 79.5 %
60 minutes exposure : 76.6 %
3 minutes exposure : 88.3 %</td><td>Anonymous 6, 2012b, Report no. 41201543</td></tr>
</table>

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
			100 µl of 0.9% w/v sodium chloride solution was added for wetting of the test item.		

Short summary and overall relevance of the provided information on skin corrosion/irritation

The active substance, ferric pyrophosphate, was tested in the study for acute dermal irritation/corrosion in rabbits. One rabbit was investigated at 3 minutes, 1 hour and 4 hours after application of the test substance immediately after the patch was removed. No evidence of a corrosive effect or symptoms of irritation were observed on the skin after application. In confirmatory test, two others rabbits were used with 4-hour exposition period. Skin reactions were evaluated for signs of erythema/eschar and oedema at 1, 24, 48 and 72 hours after exposure in all animals. No symptoms of irritation on the skin were observed. No other signs of intoxication were observed. No skin irritation was caused by 4-hour exposure of rabbits to ferric pyrophosphate. The in vitro irritating and corrosion studies have been performed using reconstituted human epidermis model. No skin irritation or corrosion hav been observed in the study.

Comparison with the CLP criteria

As a result of the test performed with Ferric Pyrophosphate, none of the criteria for skin irritancy/corrosivity classification is meet. None of the animals reached the average cut-off value of 2.3 for erythema/eschar or for oedema or in any case there was inflammatrion that persisted to the end of the observation period normally 14 days in at least 2 animals.

In in vitro study the viability of cells was ≥35% and >50% for irritation and corrosivity, respectively.

Therefore, Ferric Pyrophosphate doesn’t warrant classification as skin irritant/corrosive.

Conclusion on classification and labelling for skin corrosion/irritation

No classification in regard to acute dermal irritation/corrosion is required for ferric pyrophosphate according to criteria of the Regulation 1272/2008.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Ferric pyrophosphate was tested in three studies for skin irritation/corrosion effects.
One of the studies was carried out on rabbits according to test method B.4 in compliance with GLP.
The other two studies were carried out using a reconstituted human epidermis model with ferric pyrophosphate according to OECD TG 439 and OECD TG 431, both GLP compliant.
The skin corrosion/irritation findings did not meet the criteria for classification in any of the studies. Consequently, the DS proposed no classification for skin corrosion/irritation.

Comments received during consultation

No comments were received

Assessment and comparison with the classification criteria

Skin corrosion/irritation studies available are described in the table 14 of the CLH report.

Study 1, Anonymous 5, 2013

Ferric pyrophosphate was administrated to 3 adult female New Zealand White (NZW) rabbits. The concentration of ferric pyrophosphate was of 0.5 g for 4 hours applied onto clipped skin using a semi-occlusive dressing. The study is reliable, conducted according to Method B.4 and GLP compliant.

At first, the test substance was applied on the skin of one rabbit for 4 hours. Skin reactions were investigated 3 minutes, 1 hour and 4 hours after patch removal. No evidence of skin irritation or corrosion were observed. In the confirmatory test, no skin reactions were observed on the two additional rabbits. No other signs of intoxication were observed. Overall, no skin reactions on NZW rabbit were observed after 4h exposure to ferric pyrophosphate.

Study 2, Anonymous 6, 2012a

Ferric pyrophosphate was tested in an *in vitro* GLP compliant study performed according to OECD TG 439, and reliable. The test item was applied for 15 minutes onto the reconstituted human skin, followed by 42 hours post-exposure incubation. The average tissue viability was 110%, which is above the 50% criteria for no classification.

Study 3, Anonymous 6, 2012b

A second GLP compliant *in vitro* study was performed according to OECD TG 431 and reliable. Ferric pyrophosphate was applied directly to the reconstructed epidermis surface for 3, 60 or 240 minutes. The viability after 240 minutes was 79.5% which fulfils the prediction model for no classification (≥ 35%).

Conclusion on classification and labelling for skin corrosion/irritation

RAC agrees with the DS that **classification for skin irritancy is not warranted** for ferric pyrophosphate.

Serious eye damage/eye irritation

Table 15: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Method B.5, Council Regulation (EC) No.440/2008 GLP	Rabbit, New Zealand (albino), 3 M	Ferric pyro-phos-phate Batch 12032 7086	0.1 g, 4 hours	- No symptoms of systemic toxicity were observed in the animals during clinical observation in the test period and no mortality occurred. Weight increments were adequate to species, sex and age of animals in experiment. - Average score for each animal (mean: 24, 48, 72 h): Cornea: 0,0,0 Iris: 0, 0,0 Conjunctiva: 2,2,2 Chemosis: 0,0,0 - In two rabbits eye alterations vanished on the 5th day and in one rabbit eye alterations vanished on the 6th day after application.	Anonymous 7, 2013, Report No. 13-170
EU Method B.5 (Acute Toxicity: Eye Irritation / Corrosion) OECD Guideline 405 (Acute Eye Irritation / Corrosion)	rabbit (New Zealand White) No. of animals per sex per dose: 3	tetrairon tris(pyrophosphate) Batch number: 2-47501-56	Duration of treatment / exposure: 72 hours Observation period (in vivo): Approximately 1 hour and 24, 48 and 72 hours following treatment Amount(s) applied (volume or weight with unit): A volume of 0.1 ml of the test item, which was found to weigh approximately 98 mg	not irritating Cornea score: 0 of max. 4 (Time point: Mean 24, 48 and 72 hours) (No effects observed) (Initial pain reaction = 2) 0 of max. 4 (Time point: Mean 24, 48 and 72 hours) (No effects observed) (Initial pain reaction = 2) Iris score: 0 of max. 2 (Time point: Mean 24, 48 and 72 hours) (No effect observed) 0 of max. 2 (Time point: Mean 24, 48 and 72 hours) (No effect observed) Chemosis score: 0 of max. 4 (Time point: Mean 24, 48 and 72 hours) (No effect observed) 0.33 of max. 4 (Time point: Mean 24, 48 and 72	Anonymous 2, 2012b, Report no. 41201545
OECD 437 GLP	Tetrairon tris(pyro phosphat	Bovine eyes	-Amounts(s) applied (volume or	non-corrosive Overall irritation score (IVIS): 25.3 of max. 100 (in vitro irritancy score) (Time point:	Anonymous 6, 2012c Report no.

	e) Batch number: 2-47501-56		weight with unit): Triplicate tissues were treated - Concentration (if solution): For the purpose of this study the test item was prepared as a 20% dilution in 0.9% w/v sodium chloride solution Duration of treatment /exposure: 240 minutes.	240 minutes post-exposure) (Not applicable)	41201544

Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The active substance ferric pyrophosphate was tested in the study for acute eye irritation in rabbits. In the first study on rabbits (Anonymus 7, 2013) the following changes were observed on eye at 1 hour after application: conjunctivae – diffuse, crimson colour, individual vessels not easily discernible or diffuse beefy red and chemosis – some swelling above normal or obvious swelling with partial eversion of lids were observed in all rabbits. Diffuse, crimson colour, individual vessels not easily discernible of conjunctivae were observed in all rabbits at 24, 48 and 72 hours after application. On the 4th day some blood vessels hyperaemic (injected) of conjunctivae were observed in all rabbits. In one rabbit (No.16) this change persisted also to the 5th day. In two rabbits (No. 17 and 18) eye alterations vanished on the 5th day and in rabbit No.16 eye alterations vanished on the 6th day after application. No clinical signs of systemic intoxication were detected.
In the second study on rabbits (Anonymus 2, 2012b) no effect was observed except chemosis score 0.33 in one animal.
In a study using bovine cornea the irritation score was below of 55.1 which is defined as: “no prediction can be made”.

Comparison with the CLP criteria

Based on the result of the study by Anonymus 13 (2013): the mean scores of conjunctivae redness following grading at 24, 48 and 72 hours after installation of the test material for each of the three test animals: 2, 2, 2, classification as: irritating to eyes (Category 2) is required for ferric pyrophosphate according to criteria of the Regulation 1272/2008.

Conclusion on classification and labelling for serious eye damage/eye irritation

Ferric pyrophosphate should be classified as Eye Irrit. 2, H319 Causes serious eye irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Ferric pyrophosphate was tested in three studies for acute eye irritation, two *in vivo* studies (EU B.5 and EU B.5/OECD TG 405) and one *ex vivo* BCOP (OECD TG 437), all of which were GLP compliant. In the first *in vivo* study (Anonymous 7, 2013), an average score of 2 for conjunctival redness was observed in all 3 male of NZW rabbits. The effects were reversible within 6 days after exposure. In the second *in vivo* study (Anonymous 2, 2012b), the maximum chemosis score of 0.33 was observed in one out of 2 NZW rabbits but this adverse effect was fully reversible within 48 hours (animal no 1) and within the 72 hours (animal no 2). In the *in vitro* study (Anonymous 6, 2021c), on bovine eye, the IVIS score was 25.3 which is in the "no prediction can be made" zone, as defined in OECD TG 437 for scores between 3 < IVIS ≤55.

Based on the results of the first *in vivo* study, the DS proposed to classify ferric pyrophosphate as Eye Irrit. 2; H319, causes serious eye irritation.

Comments received during consultation

One MSCA commented on the available studies, pointing out that conjunctival redness was not investigated in Anonymous 2 (2012b), and they agreed with the DS proposal (Eye Irrit. 2; H319) based on the results of Anonymous 7 (2013).

The DS responded that additional information on the conjunctival redness results in study Anonymous 2, 2012b could be found in the REACH registration dossier (https://echa.europa.eu/registration-dossier/-/registered-dossier/12264/7/4/3): conjunctiva redness was 0.33 in two animals fully reversible within 48 and 72 hours, respectively. According to the study protocol, "if the second animal revealed corrosive or severe irritant effects, the test is not continued" and "additional animals may be needed to confirm weak or moderate irritant responses". Therefore, the results of the study by Anonymous 2 (2012b) could not be considered to be conclusive data.

A company manufacturer considered the results of the study performed by the EU REACH Registrant to be GLP and OECD TG compliant, reliable and that they clearly fulfilled the criteria for no classification. In their comment, the discrepancy between the study in the PPP review (fulfilling the criteria for Cat. 2) and these included in the REACH registration dossier could be due to a different composition, thus promoting the re-assessment of all studies based in light with accurate information on the composition. They considered the information on the EU REACH registrants did not support a harmonised classification for this endpoint.

The DS responded that the results of the *ex vivo* study (BCOP, Anonymous 6, 2012c) was 25.3 which is above the "< 3" criteria for no classification and that the other study in the REACH registration dossier could not be considered to be conclusive as only 2 animals were tested instead of 3 (Anonymous 2, 2012b). Thus, the classification is based on the results of Anonymous 7 (2013).

Assessment and comparison with the classification criteria

Study 1, Anonymous 7, 2013

Ferric pyrophosphate was administrated by instillation directly on the eyes of 3 male NZW rabbit, for 4 hours. The study was conducted accordingly to Method B.5 and was GLP compliant, and is considered reliable. One hour after instillation, chemosis and conjunctivae were observed in all rabbits, see table below. Chemosis of grade 2 was observed in all rabbits at 24, 48 and 72h after instillation, and it was completely reversible in 2 animals by day 5 and by day 6 in the third rabbit.

***Table**: CA B 6.2-4 (from RAR, 2019) Result of reaction of treated eye (grades)*

Animal No.	Ocular lesions	Time interval of examination							
		1h	24h	48h	72h	4th day	5th day	6th day	7th day
16	Cornea	0	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0
	Conjunctivae	2	**2**	**2**	**2**	1	1	0	0
	Chemosis	1	0	0	0	0	0	0	0
17	Cornea	0	0	0	0	0	0	0	-
	Iris	0	0	0	0	0	0	0	-
	Conjunctivae	3	**2**	**2**	**2**	1	0	0	-
	Chemosis	2	0	0	0	0	0	0	-
18	Cornea	0	0	0	0	0	0	0	-
	Iris	0	0	0	0	0	0	0	-
	Conjunctivae	3	**2**	**2**	**2**	1	0	0	-
	Chemosis	2	0	0	0	0	0	0	-

The mean scores of conjunctivae redness following grading at 24, 48 and 72 hours after installation were: 2, 2, 2, therefore the classification criteria for eyes irritation in Category 2 are met.

Study 2, Anonymous 2, 2012b

Ferric pyrophosphate (0.1 mL or 98 mg) was administrated by instillation directly onto the eyes on 2 male NZW rabbits for 72 hours. The study was conducted accordingly to Method B.5 and GLP compliant, and it is considered reliable.

The mean scores over time were 0.33, 0 for chemosis, and 0.33, 1 for redness. No other signs of eye irritation/corrosion were observed during the experiment.

Study 3, Anonymous 6, 2012c

Ferric pyrophosphate (20% diluted with 0.9% w/v sodium chloride solution) was applied directly to the epithelial surface of the cornea of bovine eye for 240 min (4 hours). The study was performed according to OECD TG 437, was GLP compliant, and is considered reliable.

The study irritation score was 25.3 which is in the "no stand-alone prediction can be made" range of scores ($3 < IVIS \leq 55$).

Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the result of study 1, the criteria for classification as eye irritant in Category 2 are

fulfilled, therefore RAC considers **classification as Eye Irrit. 2; H319 is warranted** for ferric pyrophosphate.

Respiratory sensitisation

Ferric pyrophosphate is used as a dietary supplement and is added to food for nutritional fortification. A respiratory sensitising potential of ferric pyrophosphate can be excluded based on the extensive experience with ferric pyrophosphate and the absence of such effect.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

DS proposed no classification for respiratory sensitisation for ferric pyrophosphate.

Comments received during consultation

No comments were received

Assessment and comparison with the classification criteria

No data are available to assess respiratory sensitisation.

Conclusion on classification and labelling for respiratory sensitisation

RAC proposes **no classification for respiratory sensitisation**.

Skin sensitisation

Table 16: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results Stimulation index	Reference
OECD 429 GLP	mouse (CBA/Ca (CBA/Ca OlaHsd)) female	iron orthophosphate Batch number: MV3395 read-across from supporting substance (structural analogue or	concentrations of 50%, 25% or 10% w/w in dimethyl formamide.	There were no deaths. No signs of systemic toxicity were noted in the test or control animals during the test. A stimulation index of less than 3 was recorded for test material at concentrations of 50%, 25% and 10% v/v in dimethyl formamide. No adverse effect observed (not sensitising). Stimulation Index (SI) Concentration [%] / SI / Result Vehicle / na / na 10 / 1.47 / negative	Anonymous 2, 2011, Report no. 41101364

		surrogate)		25	1.48	negative		
				50	2.0	negative		

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Ferric pyrophosphate is used as a dietary supplement and is added to food for nutritional fortification. A skin sensitising potential of ferric pyrophosphate can be excluded based on the extensive experience with ferric pyrophosphate and the absence of such effect.

Due to lack of skin sensitisation study for ferric pyrophosphate the data on ferric orthophosphate are used to assess their sensitisation potential. Both substances are relatively insoluble inorganic ferric (Fe^{3+}) compounds. In conditions where the substances have limited solubility/bioavailability; ionisation to the Fe cation and the orthophosphate cation (iron orthophosphate) or pyrophosphate cation (tetrairon tris(pyrophosphate) will occur. In biological systems (i.e. in the presence of alkaline phosphatase) the pyrophosphate will be broken down into orthophosphate. It is considered that the Fe^{3+} cation is of most relevance when considering the sensitisation potential of the test material and as iron orthophosphate is slightly more soluble this substance is a good candidate for read-across. Furthermore, both iron and phosphate are essential nutrients and given that humans have been exposed to iron as a nutritional supplement for many years without report of iron sensitisation potency.

Read-across is justified on the basis that the sensitisation potential of ferric pyrophosphate) will be determined by the Fe cation. Pyrophosphate itself is not considered to be a sensitiser, in addition, the breakdown product of pyrophosphate (orthophosphate) is a natural component of blood and cellular fluids. As, tetrairon tris(pyrophosphate) has a lower water solubility than iron orthophosphate, it is considered to be less bioavailable and therefore iron orthophosphate is considered to be a worst case for sensitisation potential of the Fe cation. The study reports that iron orthophosphate is a non-sensitiser under the conditions of the study.

No adverse effect observed (not sensitising)

10.7.2 Comparison with the CLP criteria

As a result of the test performed none of the criteria for skin sensitisation classification is meet. Therefore, Ferric Pyrophosphate doesn't warrant classification as skin sensitiser.

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification in regard to skin sensitisation is required for ferric pyrophosphate according to criteria of the Regulation 1272/2008.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Due to lack of skin sensitisation studies for ferric pyrophosphate, the data on ferric orthophosphate were used. Both substances are relatively insoluble inorganic ferric (Fe^{3+}) compounds. In these conditions, ionisation to the Fe cation and the orthophosphate cation (iron orthophosphate) or pyrophosphate cation (tetrairon tris(pyrophosphate)) will occur. In biological systems (i.e. in the presence of alkaline phosphatase) the pyrophosphate will be

broken down into orthophosphate. It is considered that the Fe^{3+} cation is of most relevance when considering the sensitisation potential and as iron orthophosphate is slightly more soluble, this substance is a good candidate for read-across. Pyrophosphate itself is not considered to be a sensitiser, in addition, the breakdown product of pyrophosphate (orthophosphate) is a natural component of blood and cellular fluids.

As ferric pyrophosphate has a lower water solubility than iron orthophosphate, it is considered to be less bioavailable and therefore iron orthophosphate is considered to be a worst case for sensitisation potential of ferric pyrophosphate. The study reports that iron orthophosphate is a non-sensitiser (Anonymous 2, 2011).

DS proposed no classification regarding to skin sensitisation for ferric pyrophosphate.

Comments received during consultation

No comments were received

Assessment and comparison with the classification criteria

The available study on skin sensitisation is described in Table 16 of the CLH report.

Study 1, Anonymous 2, 2011
Iron orthophosphate was administrated to female (CBA/Ca (CBA/CaOlaHsd)) mice at concentrations of 50%, 25% or 10% w/w in dimethyl formamide by direct application to calculate the Stimulation Index (SI). The LLNA study was performed according to OECD TG 429 and was GLP compliant and reliable. No mortalities or adverse effects were observed. At a 50% concentration, a value of SI = 2 was derived for iron orthophosphate, therefore the test is considered negative.

Conclusion on classification and labelling for skin sensitisation

RAC proposes **no classification as skin sensitiser** for tetrairon tris(pyrophosphate) based on the negative LLNA study conducted on the read-across substance iron orthophosphate.

Germ cell mutagenicity

Table 16: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial reverse mutation test, OECD 471 GLP	Ferric pyrophosphate Batch 120327086	Dose range finding assay: - plate incorporation method - tester strains: *Salmonella typhimurium*: TA98 and TA100 - Concentration: 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg per plate - with and without S-9 Definitive assay: - plate incorporation method - tester strains: *Salmonella typhimurium*: TA1535,	No cytotoxicity Ferric Pyrophosphate is non-mutagenic to all the five tester strains viz. TA98, TA100, TA1535,	Nikhil S. Sathe, 2014 Report No. 1248

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		TA1537 and TA102 - Concentration: 312.5, 625, 1250, 2500, 5000 µg per plate - with and without S-9 Confirmatory assay: - pre-incubation method - tester strains: *Salmonella typhimurium*: TA98, TA100, TA1535, TA1537 and TA102 - Concentration: 312.5, 625, 1250, 2500, 5000 µg per plate - with and without S-9	TA1537 and TA102 when tested at 5000 µg/plate in presence (10% v/v S9 Mix) as well as in absence of metabolic activation.	
Mammalian cell gene mutation test, OECD 476 GLP	Ferric pyrophosphate Batch 120327086	- mouse lymphoma L5178Y cell line, heterozygous at the TK locus Preliminary cytotoxicity assay: - concentrations 19.5, 39.1, 78.1, 156.3, 312.5, 625, 1250 and 2500 µg/mL - treated period 4 h - with and without S9 activation - non-activation assay with a treatment period 24 h at the same concentrations Mouse lymphoma mutagenicity assay: - concentrations 78.1, 156.3, 312.5, 625.0 and 1250 µg/mL (based on the results of preminary study and item solubility) - treated period 4 h - with and without S9 activation - non-activation assay with a treatment period 24 h at the same concentrations - concurrent negative, vehicle and positive controls both with and without S9 activation	From the results of this study and according to the criteria of the test protocol, it is concluded that when tested up to 1250 µg/mL the test item, Ferric Pyrophosphate did not induce forward mutation at the thymidine kinase (TK) locus of L5178Y mouse lymphoma cells either with or without metabolic activation under this test conditions.	Anonymus 8, 2014, Report No. VLL/1013/G/T079
In Vitro Mammalian Cell Micronucleus Test OECD 487 GLP	Ferric pyrophosphate Batch 120327086	- human peripheral blood lymphocytes Preliminary test: - concentrations: 0.05, 0.0158, 0.005, 0.00158, 0.0005 mg/ml - exposure 3 hrs in the presence or absence of S9 activation and 24 hrs without S9 activation Main study: - concentrations: 0.05, 0.0158, 0.005, 0.00158 mg/ml - exposure 3 hrs in the presence or absence of S9 activation and 24 hrs without S9 activation	The results obtained indicate that under the experimental conditons used, Ferric pyrophosphate does not induce mutagenic effect in Micronuclous test on human	Anonymus 9 2013, Report No. ZTM/2013/1/MN

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
			peripheral blood lymphocytes	

Table 17: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mammalian erythrocyte micro-nucleus test, OECD 474 GLP	Ferric pyrophosphate Batch 120327086	The aim of the study was detection of cytogenetic damages induced by the iron (III) pyrophosphate to the chromosomes or the mitotic apparatus of erythroblasts by analysis of micronuclei forming in erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals from repeated dose 90-day oral toxicity study in rodents. Ferric pyrophosphate (III) was orally administered using a stomach gavage to one group of experimental animals (one group – 15 females and 15 males), as a suspension in 0.5% methylcellulose solution in dose 1000 mg/kg b.w., once a day, in the volume max. 1 ml/100 g b.w., for 90 days, seven days a week. The control group was run in parallel and administered 0.5 % methylcellulose solution (8 females and 8 males) in the same volume as the test material. Also a positive control group (8 females and 8 males) was introduced that was administerd with ethyl methanesulphonate. All animals after dosing period were sacrificed and an autopsy was performed. During the autopsy bone marrow cells were obtained from the femurs immediately following sacrifice. Peripheral blood was obtained from heart during sacrifice. Then smear preparations from blood cells and bone marrow were made and then stained with Giemsa stain. All smear preparations were evaluated for the presence of the micronuclei.	In the study, 2000 immature erythrocytes and 2000 mature erythrocytes of bone marrow and peripheral blood were evaluated for the incidence of micronucleated erythrocytes. During analysis of slides the proportion of immature erythrocytes among mature erythrocytes of peripheral blood and bone marrow were scored by evaluated of 2000 cells. On the base of conducted study, the test material iron (III) pyrophosphate does not cause cytogenetic damages which effect forming micronuclei in the immature erythrocytes in vivo in mammals.	Anonymus 10, 2014, Report No. 0003/0030/T

Short summary and overall relevance of the provided information on germ cell mutagenicity

The mutagenic potential of ferric pyrophosphate was investigated in three *in vitro* assays (bacterial mutagenicity assay, mutagenicity test in mouse lymphoma and mutagenicity test in human peripheral blood

lymphocytes) and one *in vivo* assay (rat bone marrow micronucleus test). There were no positive results, therefore ferric pyrophosphate is not considered to be genotoxic or mutagenic in prokaryotic and eukaryotic somatic cells.

Comparison with the CLP criteria

Based on the data provided and following a weight-of-evidence approach, there is no sufficient evidence to classify ferric pyrophosphate for germ cell mutagenicity according to the CLP criteria.

Conclusion on classification and labelling for germ cell mutagenicity

No classification for germ cell mutagenicity is considered necessary, as the criteria laid down in the CLP regulation were not met.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The mutagenic potential of ferric pyrophosphate was investigated in three *in vitro* assays (bacterial mutagenicity assay, mutagenicity test in mouse lymphoma and mutagenicity test in human peripheral blood lymphocytes) and one *in vivo* assay (rat bone marrow micronucleus test). All studies were performed according to the relevant OECD TG and were GLP compliant. All results were negative, therefore ferric pyrophosphate is not considered to be genotoxic or mutagenic, consequently the DS proposed no classification for germ cell mutagenicity.

Comments received during consultation

No comments were received

Assessment and comparison with the classification criteria

All the studies regarding the mutagenicity/genotoxicity effects are summarised in the table 16 of the CLH report.

Study 1, Sathe, 2014

The GLP compliant study, performed according to OECD TG 471, is considered reliable. Ferric pyrophosphate was not mutagenic to any of the five *Salmonella* tester strains of TA98, TA100, TA1535, TA1537 and TA102 when tested at 5000 µg/plate in the presence as well as in the absence of metabolic activation (10% v/v S9 Mix).

Study 2, Anonymous 8, 2014

The GLP compliant study, performed according to OECD TG 476, is considered reliable. Due to the low solubility, the highest dose was limited by the precipitation of ferric pyrophosphate in DMSO (1250 µg/mL). No cytotoxicity and only slight precipitation were reported at this concentration. Ferric pyrophosphate did not induce any increase in the mutant frequency when tested at up to 1250 µg/mL, with or without metabolic activation, during the short or long periods of treatment; thus, it is considered not mutagenic in this system.

Study 3, Anonymous 9, 2013

The GLP compliant study, performed according to OECD TG 487, is considered reliable. The lymphocytes were isolated from two healthy, non-smoking donors.
Ferric pyrophosphate at the concentrations used (0.05 mg/mL, 0.0158 mg/mL, 0.005 mg/mL, 0.00158 mg/mL), both after 3h exposure (with or without metabolic activation system) and after 24h exposure (without metabolic activation system) did not induce any statistically significant increase in the frequency of micronuclei in exposed cell cultures compared to control cultures.

Study 4, Anonymous 10, 2014

The GLP compliant study, performed according to OECD TG 474, is considered reliable. The incidence of micronucleated erythrocytes was observed in 2000 immature erythrocytes and 2000 mature erythrocytes from the bone marrow and peripheral blood. The proportion of immature erythrocytes among mature erythrocytes of peripheral blood and bone marrow were scored and evaluated, concluding that ferric pyrophosphate does not cause cytogenetic damage which stimulates micronucleus formation in the immature erythrocytes *in vivo* in mammals.

Ferric pyrophosphate did not induce a positive response in the *in vivo* micronucleus test.

Conclusion on classification and labelling for germ cell mutagenicity

All available studies were negative, consequently, RAC proposes **no classification for ferric pyrophosphate as a germ cell mutagen**.

Carcinogenicity

Evidence for the link between iron exposure and chronic diseases is derived mainly from epidemiological studies, which have their limitations. The most important one is the lack of reliable assessment of iron intake with food and lifestyle of the participants. Most of the available studies are based on small populations, which results in low statistical power of the data obtained.

The experimental studies with multiple intravenous administration of iron in dextran conducted on mice and rats demonstrated that tumours form in the site of injection. Tests on the primates have not confirmed these observations.

Based on population observations, the link between the risk of colorectal and duodenal cancer development and iron intake with food, ferritin serum concentration or heterozygosity in hereditary haemochromatosis was studied. Results of epidemiological studies suggest that there might be a correlation between the increased iron supply (total or heme iron) and increased risk of colorectal and duodenal cancer development, however these differences were not statistically significant. Study results do not provide conclusive evidence that considerable iron overload and increased ferritin concentration might contribute to cancer development. Heterozygosity in haemochromatosis might be related to this phenomenon but this relation has also proved to be statistically insignificant. Thus, it is not possible to draw definitive conclusions. Results of studies on red meat consumption, which is a source of heme iron, invariably pointed to an increase of risk of colorectal and duodenal cancer development. However, these studies do not exclude the role of confounding variables such as environmental factors or e.g. lifestyle of the patients. It is not possible to determine the dose-effect relation and the threshold value of the amount of consumed and processed red meat.

The amount of iron that could be ingested as a result of the use of PPP containing ferric pyrophosphate in crops and ornamental plants compared to daily iron consumption with food is negligible. A chronic iron

overload as a result of the use of ferric pyrophosphate in molluscicides can be ruled out. The amount of iron which could be additionally ingested in result of the use of ferric pyrophosphate in gardens and on the filed is not relevant if compared with the amount of iron in meat and other food which is consumed daily for life.

No chronic or carcinogenicity study has been submitted for ferric pyrophosphate which was accepted for plant protection product procedure. The waiving of such a study is deemed acceptable in view of the lack of pertinent findings in genotoxicity test and repeat dose studies (up to the limit dose). No classification is proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of ferric pyrophosphate as a carcinogen.

Ferric pyrophosphate has been used as food additive for many years, even in small children. In accordance with Regulation (EU) No. 609/2013, it was approved for use in baby foods for infants and young children, processed cereal-based foods and food for children, food for special medical purposes, and in total diet replacement.

Results of epidemiological studies suggest that there might be a correlation between the increased iron supply (total or haeme iron) and increased risk of colorectal and duodenal cancer (Nelson, 2001; Torti and Torti, 2013), however these differences were not statistically significant. These study results did not provide conclusive evidence that considerable iron overload and increased ferritin concentration might contribute to cancer development. Heterozygosity in haemochromatosis might be related to this phenomenon but this relationship has also not proved to be statistically significant. Thus, it is not possible to draw definitive conclusions. Results of studies on red meat consumption, which is a source of haeme iron, invariably pointed to an increase in the risk of colorectal and duodenal cancer. However, these studies do not exclude the role of confounding variables such as environmental factors or e.g. lifestyle of the patients. It is not possible to determine the dose-response relationship and the threshold value of the amount of consumed and processed red meat.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

No carcinogenicity study was included in the CLH report for ferric pyrophosphate. The epidemiological evidence of a direct correlation between increased iron supply and colorectal and duodenal cancer is not sufficient for classification.

Conclusion on classification and labelling for carcinogenicity

RAC proposes **no classification due to lack of data**.

Reproductive toxicity

During pregnancy, physiological changes in the organism of a pregnant woman cause a decrease in the level of hemoglobin, which may lead to anemia. To meet iron demand increased by 1000 mg, stored iron is released in the organism, but dietary fortification or even supplementation are also indicated. Ferric pyrophosphate is one of iron sources approved in the European Union for food fortification and dietary supplement. No premises suggesting the substance used orally might potentially have a toxic effect on germ cells and reproduction are known of, and the risk related to its use in plant protection products can be ruled out. WHO report cites the results of studies on the influence of iron and its compounds on reproduction, which show that no maternal toxicity or teratogenic effects were observed for doses up to 160 mg/kg bw in mice and rats (ferric sodium pyrophosphate).

No reproductive toxicity study has been submitted for ferric pyrophosphate which was accepted for plant protection product procedure. The waiving of such a study is deemed acceptable in view of the use of ferric pyrophosphate as dietary supplement and for nutritional fortification. No classification is proposed.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification for reproductive toxicity of ferric pyrophosphate.

During pregnancy, iron demand is significantly increased and dietary fortification or supplementation are indicated. Ferric pyrophosphate is one of iron sources approved in the European Union for food fortification and dietary supplement.

A WHO report[1] cites the results of studies on the influence of iron and its compounds on reproduction, which show that no maternal toxicity or teratogenic effects were observed for doses up to 160 mg/kg bw/d in mice and rats (ferric sodium pyrophosphate).

Despite this, some studies describe potential correlation of iron overload with birth and infant adverse health outcomes including growth retardation, foetal malformations or preterm births. The level of evidence is, however, rather low due to the limited sample size[2]. In another study, patients with beta-thalassemia experienced iron overload and impaired fertility[3]. In these patients sexual maturation was delayed and they had hypogonadism and sperm DNA damage. This could be due to the potential for iron to increase ROS production, decrease antioxidant levels, enhance the lipid peroxidation of the cell membrane, cause apoptosis, and contribute to the oxidative damage of DNA[4,2].

Comments received during consultation

No comments were received

Assessment and comparison with the classification criteria

1 Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain food additives and food contaminants. WHO Food Additives Series, No. 18, 571. Iron; 1983

2 Brannon and Taylor. Iron Supplementation during Pregnancy and Infancy: Uncertainties and Implications for Research and Policy, Nutrients; 2017, 9, 1327

3 Golub (ed) (2006) Metals, fertility, and reproductive toxicity. Taylor and Francis, Boca Raton

4 Pizent *et al.* Reproductive toxicity of metals in men, Arh Hig Rada Toksikol; 2012; 63

No reproductive toxicity study has been submitted for ferric pyrophosphate.

The available epidemiological information does not indicate adverse effects of ferric phosphate on reproductive hazard for humans or mammals.

Conclusion on classification and labelling for reproductive toxicity

RAC proposes **no classification due to lack of data**.

Specific target organ toxicity-single exposure

Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

See section 10.1-10.3 for results of acute toxicity studies. The substance was administered in single dose toxicity studies (limit dose) by oral and inhalation routes which are designed to investigate mortality effects and LD/LC_{50} setting. Notwithstanding, no adverse effects were mentioned that can be relevant to humans i.e. that can impair function, reversible or irreversible, immediate and/or delayed.

During the acute toxicity study by oral route the animals were examined for clinical changes in areas such as: locomotor system, behaviour, reactions to stimuli, skin and hair, eyes and eyelids, respiratory system, digestive system, urinary system, reproductive system, whereas in the acute inhalation study observations included, but were not be limited to: changes in the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behavior pattern. Attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and rectal temperaturę. No non-lethal effects were reported after acute exposure of ferric pyrophosphate via oral and inhalative route, including clinical signs, influence on behaviour, effects on body weight gain or changes in macroscopic examination. It is not anticipated that ferric pyrophosphate has specific target organ toxicity, under single-dose exposure. No known mechanisms of narcotic effects are expected to occur in case of ferric pyrophosphate based on its molecular structure, solubility and potential mode of action. Ferric pyrophosphate has been used as food additive for many years, even in small children. In accordance with Regulation (EU) No. 609/2013 of the European Parliament and of the Council of 12 June 2013, it was approved for use in baby food for infants and young children, processed cereal-based foods and food for children, food for special medical purposes, and total diet replacement. There is no evidence of RTI effect of the substance, however its potential mechanism would be associated with physical/mechanical irritation during dust inhalation, what according to the Guidance on the Application of the CLP Criteria precludes the classification. Based on extensive experience with the substance neither narcotic effects nor cause-relatated RTI are reported.

Comparison with the CLP criteria

No single dose toxicity studies other than acute limit tests were submitted to allow the assessment of non-lethal toxic effects.

Conclusion on classification and labelling for STOT SE

Considering that no non-lethal effects were reported after acute exposure, no hazard classification is proposed.

RAC evaluation of specific target organ toxicity – single exposure

(STOT SE)

Summary of the Dossier Submitter's proposal

Ferric pyrophosphate was investigated in a number of acute studies by the oral and inhalation routes (see section 10.1-10.3 of CLH report). There was no indication that ferric pyrophosphate caused specific toxicity to any organ after a single exposure. There was no evidence of narcotic effects from any toxicological study. No signs of respiratory irritation were observed in the acute inhalation study.
The DS did not consider a classification for specific target organ toxicity – single exposure (STOT SE) for ferric pyrophosphate appropriate based on the findings from the acute toxicity studies.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

During the acute oral toxicity study the animals were examined for clinical changes in areas such as: locomotor system, behaviour, reactions to stimuli, skin and hair, eyes and eyelids, respiratory system, digestive system, urinary system, reproductive system, whereas in the acute inhalation study observations included, but were not be limited to: changes in the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system, somatomotor (pertaining to movements of the body) activity and behaviour pattern. Attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and rectal temperature.

Non-lethal effects were not reported after acute exposure to ferric pyrophosphate via oral or inhalation route, including clinical signs, influence on behaviour, and effects on body weight gain or changes in macroscopic examination. It is not anticipated that ferric pyrophosphate has specific target organ toxicity, under single-dose exposure.

No known mechanisms of narcotic effects are expected to occur in case of ferric pyrophosphate based on its molecular structure, solubility.

Ferric pyrophosphate has been used as food additive for many years, even in small children. In accordance with Regulation (EU) No. 609/2013 of the European Parliament and of the Council of 12 June 2013, it was approved for use in baby food for infants and young children, processed cereal-based foods, food for children, for special medical purposes, and as total diet replacement. In principle, ferric pyrophosphate could cause respiratory tract irritation (RTI) through a physical/mechanical irritation mode of action following dust inhalation. However, no evidence of irritation was observed in the acute toxicity study. Furthermore, no narcotic effects nor cause-related RTI have been reported following extensive experience with the substance.

Conclusion on classification and labelling for STOT SE

No single dose toxicity studies other than acute limit tests were submitted to enable the assessment of non-lethal toxic effects. Despite all the studies, even at the limit dose, no signs

which could indicate specific effects on target organs were reported.

Overall, RAC concludes that **no classification for STOT SE is warranted** for ferric pyrophosphate.

Specific target organ toxicity-repeated exposure

Table 18**: Summary of repeated dose toxicity studies**

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
OECD 407, deviation: differential leukocyte formula assay and the number of reticulocytes in peripheral blood (peripheral blood image) were not performed. Rat, Wistar 6 F + 6 M GLP	Ferric pyrophosphate Batch 120327086 Oral in feed, 28 days 0, 100, 500, 1000 mg/kg bw/day	There were no clinical signs of toxixity. There were no statistically significant changes in terms of all parameters, in comparison with the control group. Additionally, all parameters were in the range of reference standards. There were no deaths. The body weights of all animals were within the reference ranges for Han Wistar rats. There were no statistically significant differences between animals 1000 mg/kg body weight and the control group. Fodder consumption did not differ from the reference values. Water consumption was also within range of reference standards. Regarding blood analysis all values were within the range of reference values. Organ weights taken as an anatomical specimen in the study group did not differ significantly from the control group animals. There were no pathological changes in macroscopic examination.	Anonymus 11, 2013, Report No. 0003/0016/T

OECD 408, deviation: different rat strain was used in this study than in 28 days oral study, the parameters of coagulation had not been evaluated for all animals and some organs were taken in the form of an anatomical preparation without dissection of individual organs. Rat, Wistar Experimental group 15 F + 15 M; Control group 8 F + 8 M; Satelite group 8 F + 8 M GLP	Ferric pyrophosphate Batch 120327086 Oral in feed, 90 days 0, 1000 mg/kg bw/day	There is no evidence of toxicity caused by the action of ferric pyrophosphate, what was confirmed by haematological, biochemical and histopathological test, as also analysis of behavioral and neurological disorders. There were no treatment-related clinical signs at any dose. There were no deaths. The body weights of all animals were within the reference ranges for Wistar rats. There were no statistically significant differences between animals 1000 mg/kg body weight and the control group. Food consumption by animals participating in the study did not differ from the reference values. Water consumption also ranged in reference standards. Pathologic discharge from reproductive organs – absence. There were no pathological changes in macroscopic examination.	Anonymus 12, 2014, Report No. 0003/0017/T

Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

There were no deaths and clinical signs of toxicity following 28 days oral exposure to ferric pyrophosphate. Hematological parameters did not show statistically significant differences between the exposed groups and the control. Statistically significant differences as higher values of alanine aminotransferase, aspartate aminotransferase and potassium concentration in the control group in comparison to the group exposed to 1000 mg/kg bw were within the reference values. No signs of toxicity related to elevated enzymes and potassium level were noted. Patomorphological analysis of liver of animals did not reveal hepatic disfunction. Macroscopic examination indicated no pathological changes in the tested organs.

There were no deaths and clinical signs of toxicity following 90 days oral exposure to ferric pyrophosphate. Hematological parameters showed statistically significant increase of leukocytes and reticulocytes in exposed females and red blood cell counts and hematocrit in exposed male and female. These changes were within the reference values and did not correlate with other clinical symptoms. The analysis of plasma and serum revealed statistically significant increase of the following parameters measured in exposed females: unsaturated iron binding capacity UIBC, phosphorus, triglycerides, urea, total protein and albumin. The level of glucose and total cholesterol were decreased in exposed females.
In case of males the following parameters were higher in exposed group: level of magnesium, iron, urea, creatinine, alanine aminotransferase, alkaline phosphatase and amylase. Other parameters as phosphorus, total iron binding capacity TIBC and unsaturated iron binding capacity UIBC were lower in comparison to control. These changes were within the reference values if they existed. In other case these alterations could not be associated with the iron pyrophosphate influence as they were slight and the standard deviations were large. The autopsy demonstrated no pathological changes in the tested organs.

Potential exposure, other than oral, is very limited. This is related to the physicochemical properties of the substance - it is insoluble in water, lipids and organic solvents, which makes the transdermal exposure extremely low. The compound has a form of non-volatile powder, which is supposed to be added to PPP as a solid - granules, whose size prevents absorption via inhalation.

Table 19. The results of haematology examinations of female and male rats exposed to ferric pyrophosphate for 90 days at dose of 1000 mg/kg bw/d

	Control group		1000 mg/kg bw/day for 90 days		1000mg/kg bw/day for 90 days with 14 days recovery period (satellite group)	
Parameter	Females	Males	Females	Males	Females	Males
	Morphology					
WBC [thous./µl]	5,1 ± 1,5 N=8	6,3 ± 0,7 N=8	8,9 ± 6,1 N=13	5,5 ± 0,9 N=13	4,5 ± 1,5 N=8	4,9 ± 1,1 N=8
RBC [mln/µl	7,2 ± 0,6 N=8	8,0 ± 0,3 N=8	8,1 ± 1,0 N=13	8,4 ± 0,4 N=13	7,8 ± 1,3 N=8	8,2 ± 1,3 N=8
HGB [g/dl]	19,9 ± 14,9 N=8	15,6 ± 0,4 N=8	16,2 ± 2,1 N=13	15,9 ± 0,6 N=13	15,4 ± 2,0 N=8	15,7 ± 2,2 N=8
HCT [%]	40,0 ± 3,2 N=8	42,2 ± 1,5 N=8	45,1 ± 5,8 N=13	44,2 ± 2,0 N=13	43,1 ± 6,5 N=8	43,7 ± 6,9 N=8
MCV [fl]	55,8 ± 2,1 N=8	52,8 ± 1,3 N=8	55,5 ± 1,3 N=13	52,8 ± 1,0 N=13	55,6 ± 1,4 N=8	53,3 ± 1,3 N=8
MCH [pg]	20,6 ± 1,1 N=8	27,3 ± 21,5 N=8	19,9 ± 0,5 N=13	19,0 ± 0,4 N=13	19,9 ± 1,1 N=8	19,1 ± 0,5 N=8
MCHC [g/dl]	36,9 ± 0,9 N=8	37,1 ± 0,7 N=8	35,3 ± 1,1 N=13	35,9 ± 0,6 N=13	35,8 ± 1,5 N=8	36,0 ± 0,7 N=8
PLT [thous./µl	806,8 ± 53,4 N=8	824.9 ± 77,3 N=8	829,1 ± 57,3 N=13	834,5 ± 67,2 N=13	683,5 ± 162,2 N=8	626,3 ± 217,5 N=8
Reticulocytes [part-per-thousand]	19,6 ± 9,5 N=8	34,9 ± 18,8 N=8	39,6 ± 7,8 N=13	23,2 ± 7,5 N=13	44,8 ± 19,3 N=8	48,1 ± 19,3 N=8
	Coagulation parameters					
APTT	20,6 ± 6,1 N=7	17,7 ± 0,8 N=8	18,1± 1,7 N=14	16,9 ± 2,0 N=12	18,3 ± 1,4 N=8	18,4 ± 1,8 N=8
INR	0,8 ± 0,0 N=7	0,9 ± 0,0 N=8	0,8 ± 0,0 N=14	0,9 ± 0,0 N=12	n/d	n/d
PT	11,1 ± 0,4 N=7	11,8 ± 0,5 N=8	11,1± 0,3 N=14	11,8 ± 0,4 N=12	10,0 ± 0,0 N=8	10,1 ± 0,4 N=8
WSK. PT (PR)	123,2 ± 3,3 N=7	118,3 ± 2,2 N=8	123,3 ± 3,2 N=14	115,3 ± 2,9 N=12	n/d	n/d
TT	20,6 N=1	28,9 ± 3,5 N=6	33,3 N=1	26,3 ± 3,1 N=9	23,6 ± 1,9 N=8	26,6 ± 2,1 N=8
	Microscopic examination of bone marrow					
Red blood cells system [%]	32,0 ± 3,4 N=8	34,3 ± 3,6 N=8	31,4 ± 3,6 N=14	31,4 ± 2,1 N=13	33,8 ± 3,2 N=8	32,8 ± 2,7 N=8
Granulocytic system [%]	56,8 ± 3,9 N=8	54,6 ± 4,5 N=8	58,1 ± 5,3 N=14	57,9 ± 3,3 N=13	55,3 ± 3,5 N=8	56,3 ± 3,5 N=8
Lymphocytes [%]	11,3 ± 1,2 N=8	11,1 ± 1,1 N=8	10,6 ± 1,7 N=14	10,7 ± 1,3 N=13	11,0 ± 0,9 N=8	11,0 ± 1,2 N=8

	Microscopic examination of peripheral blood Percentage of white blood cell types					
Banded Neutrophils [%]	1,9 ± 1,1 N=8	0,9 ± 0,4 N=8	1,5 ± 0,7 N=14	1,8 ± 0,4 N=13	1,8 ± 0,7 N=8	1,5 ± 0,5 N=8
Segmented Neutrophils [%]	24,4 ± 2,9 N=8	26,8 ± 4,0 N=8	24,2 ± 4,1 N=14	24,2 ± 2,7 N=13	25,3 ± 3,0 N=8	27,3 ± 3,2 N=8
Eosinophils [%	1,1 ± 0,4 N=8	1,1 ± 0,4 N=8	1,1 ± 0,3 N=14	1,1 ± 0,3 N=13	1,1 ± 0,4 N=8	1,4 ± 0,5 N=8
Basophils [%]	0,1 ± 0,4 N=8	0,3 ± 0,5 N=8	0,1 ± 0,4 N=14	0,1 ± 0,3 N=13	0,1 ± 0,4 N=8	0,1 ± 0,4 N=8
Lymphocytes [%]	70,8 ± 2,3 N=8	69,4 ± 4,4 N=8	70,9 ± 4,7 N=14	70,8 ± 2,8 N=13	69,8 ± 3,2 N=8	67,8 ± 2,7 N=8
Monocytes [%]	1,8 ± 0,5 N=8	1,6 ± 0,7 N=8	2,1 ± 0,9 N=14	2,1 ± 0,8 N=13	2,1 ± 0,8 N=8	2,0 ± 0,5 N=8

WBC (leukocytes) – white blood cells count, RBC – red blood cells count,HGB – haemoglobin, HCT – haematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration, PLT – platelet count, APTT – activated partial thromboplastin time, INR – international normalized ratio, PT – prothrombin time, WSK. PT – prothrombin ratio, TT – thrombin time

Table 20. Mean values and standard deviation values of all biochemistry parameters for each group

Number of animals in groups	Dose / Group	Sex	Mean ±SD	Sodium[mmol/l]	Potassium [mmol/l]	Cholesterol[mg/dl]	Triglicerides[mmol/l]	Creatinine [mg/dl]	Urea [mg/dl]	Aspat [U/l]	Alat [U/l]	Total bilirubine [mg/dl]	Albumines [g/dl]	Amylase [U/l]	Total protein [g/dl]
14	1000	♀	Mean	143,07	5,44	66,14	5,24	5,41	56,57	94,36	51,43	1,85	3,39	893,50	6,01
		♀	SD	1,14	0,64	6,89	16,05	17,73	9,03	12,98	9,59	6,09	0,14	166,90	0,23
13	1000	♂	Mean	143,92	5,78	76,69	0,56	0,62	48,38	98,62	46,08	0,19	3,26	1780,92	6,23
		♂	SD	2,72	0,33	9,87	0,10	0,03	9,18	17,33	15,18	0,02	0,14	175,75	0,22
8	satellite group	♀	Mean	143,50	5,76	84,50	0,61	0,63	39,38	117,50	51,50	0,25	3,28	1038,38	5,81
		♀	SD	2,00	0,43	12,71	0,09	0,04	9,66	46,60	26,25	0,03	0,12	121,19	0,26
8	satellite group	♂	Mean	142,38	5,81	74,38	0,50	0,54	35,25	107,38	24,75	0,21	3,23	1308,00	5,99
		♂	SD	1,41	0,49	9,69	0,21	0,02	3,37	10,38	2,38	0,02	0,09	183,96	0,10
8	control group	♀	Mean	142,63	5,19	87,50	0,60	0,68	41,75	104,25	45,38	0,21	3,23	994,63	5,71
		♀	SD	1,92	0,38	9,65	0,17	0,05	4,68	19,90	12,36	0,02	0,07	139,22	0,13
8	control group	♂	Mean	145,25	6,25	85,25	0,65	0,57	36,13	106,00	27,63	0,18	3,21	1334,63	6,12
		♂	SD	1,39	0,92	12,16	0,23	0,04	3,18	17,11	7,42	0,01	0,10	145,31	0,19

Number of animals in groups	Dose / Group	Sex	Mean ±SD	Glucose [mg/dl]	Alkaline Phosphatase [U/L]	GGTP [U/l]	Lipase [U/l]	Magnesium [mmol/l]	TIBC [µmol/l]	Total calcium [mmol/l]	Ferrum [ug/dl]	Posfor [mmol/l]	Chlorides mmol/l]	Ferritine [ng/ml]	Bile acids [umol/l]	UIBC [µmol/l]	AcCh [µmol/dm 3]
14	1000	♀	Mean	150,93	48,57	6,00	5,00	1,24	491,14	11,25	286,86	21,06	103,43	253,85	88,21	216,09	0,40
		♀	SD	36,75	7,43	0,00	0,00	0,08	32,07	1,17	67,45	70,50	1,70	33,73	42,37	84,51	0,12
13	1000	♂	Mean	186,38	73,15	6,00	5,00	1,21	489,08	11,35	164,08	2,51	103,00	290,85	38,62	332,04	0,39
		♂	SD	26,74	10,61	0,00	0,00	0,06	40,21	0,36	32,25	0,21	2,42	49,09	21,74	33,27	0,11
8	satellite group	♀	Mean	177,13	49,63	6,00	5,38	1,25	473,63	11,49	326,63	2,18	107,00	243,57	60,33	139,50	0,43
		♀	SD	47,11	12,21	0,00	0,80	0,15	26,77	0,15	41,13	0,30	2,33	109,74	31,82	50,22	0,12
8	satellite group	♂	Mean	185,38	49,38	6,00	5,03	1,04	498,25	11,69	115,75	3,11	101,75	290,33	22,60	383,35	0,24
		♂	SD	32,29	8,53	0,00	0,08	0,05	28,29	0,29	12,42	0,43	1,04	45,53	19,25	27,10	0,10
8	control group	♀	Mean	196,13	46,75	6,00	5,22	1,36	430,88	11,22	289,75	2,30	105,75	228,50	69,48	164,29	0,44
		♀	SD	22,26	9,39	0,00	0,53	0,11	35,87	0,25	31,12	0,31	1,28	57,47	36,97	51,95	0,10
8	control group	♂	Mean	186,63	50,13	6,00	5,53	1,15	501,13	11,81	109,25	3,09	104,25	295,47	21,81	394,90	0,39
		♂	SD	42,38	9,80	0,00	0,77	0,14	19,27	0,52	11,51	0,44	1,49	42,28	18,13	19,71	0,17

Comparison with the CLP criteria

No severe findings with significant organ damage were observed in rats at dose levels below the respective guidance values in oral route. Hence, it is proposed not to classify for STOT RE.

Conclusion on classification and labelling for STOT RE

Classification for effects seen in repeated-dose studies was considered not necessary.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Two studies were performed on Han Wistar or Wistar rats according to OECD TG 407 and OECD TG 408, respectively, with deviations. Tetrairon tris(pyrophosphate) was administrated by feeding for 28 days or 90 days. The scope of these studies was to identify the long-term effects during repeated exposure on animals.

The DS did not propose to classify ferric pyrophosphate as STOT RE either in category 1 or 2.

Comments received during consultation

No comments were received

Assessment and comparison with the classification criteria

The studies are presented in Table 18 of CLH report.

Study 1, Anonymous 11, 2013

Ferric pyrophosphate was administered by gavage to Han Wistar rats in concentrations of 0, 100, 500, 1000 mg/kg bw/d as a suspension in 0.5% methylcellulose solution, in the same volume (1 mL), for 28 days (7 days/week). The was performed according to OECD TG 407, GLP compliant and reliable.

No mortalities or clinical signs of toxicity were noted. Haematological parameters and serum biochemistry either did not show statistically significant differences between the exposed groups and the control, or were within the reference values. No signs of toxicity related to elevated enzymes and potassium level were noted. Gross pathology findings did not indicate systemic toxicity of ferric pyrophosphate up to 1000 mg/kg bw/d.

Study 2, Anonymous 12, 2014

Ferric pyrophosphate was administrated by gavage to 15/sex Wistar rats for 90 days, at a concentration of 1000 mg/kg bw/d, suspended in 0.5% methylcellulose solution seven days a week. Concurrently, control group (8 males/females) received the vehicle (0.5% methyl

cellulose solution) at the same volume as the test material, and a satellite group (8 males/females) received the test material at a dose of 1000 mg/kg bw/d.

The study is considered reliable, was performed according to OECD TG 408 and was GLP compliant. The study deviates from the original planned study because a different rat strain was used in this study than in 28 day oral study, the coagulation parameters were not evaluated for all animals.

No mortalities or clinical signs of toxicity were noted and the body weight, food and water consumption, and pathology were normal. From the 11th week, behavioural tests were carried out and on the last exposure day the animals underwent neurological examination.

The statistically significant changes in haematological and biochemical parameters in the exposed animals were within reference values provided by the applicant for rats, and they do not constitute evidence of severe adverse effects caused by ferric pyrophosphate.

Results

Blood analysis

Statistically significant haematological findings were increased red blood cell count and haematocrit value in male and female tested group (1000 mg/kg bw/d) in comparison with the control group, while leukocytes and reticulocytes were increased in females only. These findings did not exceed the reference values and did not correlate with other clinical symptoms.

Plasma and serum analysis

Statistically significant differences in females were increased unsaturated iron binding capacity, phosphorous, triglycerides, urea, albumin and total protein level, and decreased glucose and total cholesterol levels. In males, statistically significant changes were as follows: increased magnesium, iron, urea, creatinine, alanine aminotransferase, alkaline phosphatase and amylase levels. In addition, statistically significant decreased phosphorous levels, as well as unsaturated and total iron binding capacities were reported in males. These statistically significant biochemical differences were within the available reference values and did not correlate with other clinical symptoms. Pathologic discharge from reproductive organs was not included in the CLH dossier.

Conclusion on classification and labelling for STOT RE

No significant organ damage was observed in rats up to 1000 mg/kg bw/d. Consequently, RAC agrees with the DS's proposal for **no classification**.

Aspiration hazard

Ferric pyrophosphate is not a hydrocarbon and is not known to cause human aspiration toxicity hazards. Therefore, no classification is warranted for aspiration toxicity.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification warranted for aspiration toxicity.

Ferric pyrophosphate is not a hydrocarbon and is not known to cause human aspiration toxicity hazards.

Comments received during consultation

No comments were received

Assessment and comparison with the classification criteria

RAC agrees with the DS assessment of aspiration toxicity.

Conclusion on classification and labelling for aspiration toxicity

RAC proposes **no classification**.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Rapid degradability of organic substances

Not applicable. Ferric pyrophosphate is an inorganic substance.

Environmental transformation of metals or inorganic metals compounds

Ferric pyrophosphate - is a stable non-volatile inorganic salt, virtually insoluble in water. On the other hand, its components - iron and phosphorus - are elements naturally occurring in both the terrestrial and aquatic environments.

Iron is the second most abundant metal in the natural environment and the fourth most abundant element, which composes about 5% of the Earth's crust. In the environment, it is found in the form of minerals such as: hematite, magnetite, siderite or pyrite. The content and distribution of iron in soils varies but typically it is 1–5% (10 – 50 g/kg). Heavy soils might sometimes contain twice as much iron as sandy soils. Most of the iron in soil is found in silicate minerals or iron oxides and hydroxides, forms that are not readily available for plant use. Examples of iron phosphates found in soil are vivianite, stable in anaerobic conditions ($Fe_3(PO_4)_2$ x 8 H_2O) and strengite, stable in acidic soils ($FePO_4$ x 2 H_2O). Iron is one of the most mobile elements in soil and in unfavourable conditions it very fast moves deep into the soil profile, which decreases the amount of forms readily available for plant use. Iron compounds are released as a result of soil or rock weathering. Under typical environmental conditions, the element is found in two oxidation states - reduced, as ferrous ion Fe^{2+}, or oxidized, as ferric ion Fe^{3+}. Even though most of iron in the Earth's crust has the ferric form Fe^{3+}, it is the ferrous form Fe^{2+} that is more physiologically important for plants. This form is relatively soluble but it is readily oxidized to Fe^{3+}, which precipitates as very insoluble oxides and hydroxides and thus becomes inaccessible to plants. Soil pH and the aeration status of the soil determine which form predominates. Ferric compounds (Fe^{3+}) have low solubility in the soil solution, and conditions that favour formation of these compounds decrease iron availability. The concentration of iron in the soil solution decreases sharply as the soil pH increases. Iron content in edible plant organs is 10 - 320 mg/kg of dry weight. The element is

essential for the production of chlorophyll, it is found in certain proteins and takes part in the process of cellular respiration. Deficiency symptoms, manifested as leaf chlorosis, appear first on the youngest leaves but with time they can also affect older leaves. To cope with low iron availability in soil, plants have developed various mechanisms for iron acquisition. One of them is excreting hydrogen ions (H^+) from roots, which lowers the pH at the root surface and increases the solubility of iron. Another mechanism is the release of ferric ion chelating agents - siderophores - which by forming complexes with Fe^{3+}, increase their solubility.

Phosphorus is an element essential for the functioning of every cell. It is a component of many important compounds such as nucleic acids and ATP - a key compound in intracellular energy transfer. Phosphorus is found in soil in two forms: organic and mineral. The main inorganic forms of phosphorus are phosphate ions solved in water $H_2PO_4^-$ and HPO_4^{2-}. In the soil solution of pH 4.5-7.0, phosphorus occurs mainly as $H_2PO_4^-$ ions, which are directly absorbed by roots, and in alkaline soils as HPO_4^{2-}. These ions react readily with iron, aluminium, and manganese compounds in acid soils and with calcium compounds in neutral and alkaline soils, forming compounds which plants cannot assimilate. Due to the adsorption on the surface of the solid phase of soil and formation of insoluble phosphate precipitates, they become inaccessible to plants. About 15-80% of phosphorus in soil is found in organic compounds (nucleic acids, phospholipides, phytate) from plant residues. Phosphorus resources in soil are scarce and its total concentration ranges between 50 and 3000 mg of phosphorus/kg (or 275 – 16 500 mg/kg expressed as pyrophosphate $P_2O_7^{4-}$). Phosphorus compounds in soil display great diversity both in terms of chemical forms and the strength of bonding with the solid phase of soil. One of the unique characteristics of phosphorus is its immobility in soil. Apatite is the main source of phosphorus in soil.

Mineral nutrients absorbed by plants are one of the environmental factors essential for plant growth and development. Proper mineral metabolism is of key importance for optimum yields. Certain elements, like Fe, undergo rapid oxidation and precipitation in soil. Thus, plants do not use them effectively. In order to prevent these processes, chelated fertilizers, in which a metal nutrient ion is combined with a chelator, are used. An element encircled by the chelator does not degrade in soil, does not form poorly soluble compounds and is easily absorbed by plants. In agriculture, several chelating agents are allowed for use, e.g. EDTA, which prevents the conversion of Fe^{2+} to Fe^{3+}. Doses of chelated fertilizers containing 6-12% Fe suggested by the manufacturers are usually about 0.6 – 2.2 kg of iron/ha. Approximate doses of fertilizer containing 6 - 7% Fe, recommended in garden plant cultivation are as follows: 0.6 kg Fe/ha as preventive measure, 3 kg Fe/ha in the case of moderate deficiency and 6 kg Fe/ha in the case of serious deficiency. By way of comparison, the amount of iron, added to soil after a single application of plant protection product containing ferric pyrophosphate in the amount of 50 kg/h is 13 times lower than the fertilizer dose used in the case of severe iron deficiency and amounts to 0.45 kg Fe/ha.

In phosphorus fertilizers, about half of phosphorus has the form of orthophosphate and the remaining phosphorus is condensed mainly as pyrophosphate. What decides about pyrophosphate being an effective source of phosphorus in a fertilizer is the speed of its hydrolysis to the orthophosphate form, which is caused almost solely by catalysis via pyrophosphatase with the presence of divalent metal ions. The hydrolysis depends on many factors such as biological activity, water content, pH and temperature. In warm wet soils, polyphosphate ions react with soil moisture to form orthophosphates relatively rapidly (1–2 weeks), whereas in cool and dry conditions, hydrolysis might proceed more slowly. Since practically all soluble phosphorus from fertilizer or manure is converted in the soil to water-insoluble phosphorus within a few hours after application, the use of polyphosphate fertilizers is more effective. This stems from the fact that polyphosphate compounds are less reactive in soil compared to orthophosphates and thus less prone to precipitation, which might increase availability of phosphorus in soil and its uptake by plants. Moreover, it is claimed that polyphosphates are superior to orthophosphates because they have an ability to chelate and combine with certain micronutrients (e.g. Zn) and hold them in an available form. The average use of phosphorus fertilizers in Poland in the years 2011/2012 was 24.8 kg P_2O_5/ha of arable land. By way of comparison, the amount of phosphates, expressed as P_2O_5, introduced to soil after a sixfold application of plant protection product containing ferric pyrophosphate in the amount of 50 kg/h per application is 4.8 times lower than the average annual dose of phosphorus fertilizers used in Poland and amounts to 5.13 kg P_2O_5/ha.

In accordance with the document of the United States Environmental Protection Agency, no unfavourable ecological and environmental effects of using iron salts as plant protection products have been identified. It is not expected that iron salts present in plant protection products or fertilizers will affect in any significant way the fate of compounds naturally occurring in the environment. As a result of using iron salt, ferric

oxides and hydroxides are formed, which are in no way different from those naturally occurring in soil and which are responsible for its brown and red colour. Both iron and phosphorus are natural components of soil and key nutrients for plants and animals. The amount of ferric pyrophosphate added as a result of application compliant with GAP will be negligible compared to the amount naturally occurring in the environment. As for toxicity to man and ecotoxicity, there are no specific concerns about the fate and behaviour of ferric pyrophosphate in soil after application compliant with GAP, thus no studies on fate and behaviour in soil were conducted.
Iron salts, iron and phosphorus naturally occur in aquatic ecosystems. Inorganic iron and phosphorus ions do not degrade and comprise a natural fertilizer for algae and plants. In moderate and high temperatures, increased level of phosphorus in surface waters causes eutrophication i.e. explosive growth of algae accompanied with a decrease in dissolved oxygen. However, it is not expected that the natural amount of iron and phosphorus in surface waters and sediment will be significantly changed as a result of using plant protection product containing ferric pyrophosphate in accordance with the rules of good agricultural practice and label information.
The justification for waiving the environmental fate and behaviour studies was acceptable for plant protection product procedure.

Ferric pyrophosphate data from transformation/dissolution test according to the OECD TG 29 is not available. Therefore, the analysis of transformation could be based on the read-across data for iron ortophosphate. Iron ortophosphate is a ferric phosphate salt, composed of a phosphate as anion ($PO4^{3-}$) and iron as cation (Fe^{3+}). Taking into account the similar structure, physical-chemical properties, environmental fate properties and ecotoxicological profile of substances, data of iron orthophosphate can be used. The 28d transformation/dissolution test according to the OECD guideline 29, from REACH registration dossier for iron ortophosphate, determined a maximum dissolution of 21.062 µg/L iron species after 7 d at a loading of 100 mg/L and pH 6, indicating that soil and sediment are expected to be the primary environmental compartments of relevance for the substance. Furthermore, no concerns from bioaccumulation are expected, since both elements iron and phosphorous are essential elements for life and the releases of the metals from the substance are very low. Since the substance is inorganic the biodegradation concept does not apply.

Environmental fate and other relevant information

Not relevant. All information is reported under chapter 11.2.

Bioaccumulation

Since ferric pyrophosphate is insoluble in water, octanol/water partition coefficient cannot be established. However, the risk of bioaccumulation can be ruled out due to the natural occurrence of iron and phosphorus in the environment in both the aquatic ecosystem and all living organisms and the key role of these elements in the metabolism of plants and animals. They are indispensable for their proper functioning and for metabolic processes, and their amount absorbed from food is strictly regulated. In addition, ferric pyrophosphate, used also as a dietary supplement and food additive, is insoluble in organic solvents, thus its bioconcentration in organisms is not expected.

Conclusion

There is low potential for bioaccumulation of ferric pyrophosphate.

Acute aquatic hazard

The summary of the acute aquatic toxicity studies of ferric pyrophosphate is reported below. Only information considered adequate, reliable and relevant for the classification proposal has been included.

Table 21: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Acute toxicity to rainbow trout OECD 203	rainbow trout (*Oncorhynchus mykiss*)	Ferric pyrophosphate Batch 120327086	LC_{50}> 0.134 mg/L (measured concentration; solubility limit) – LC_{50} > 100 mg/L (nominal concentration)	Exposure: 96 h, static Measured and nominal concentration 14,40°C - 16,10 °C pH 8,5	Anonymous 13 (2013); Report No. 0003/0024/E
Aquatic invertebrates short-term toxicity OECD 202	*Daphnia magna*	Ferric pyrophosphate Batch 120327086	48h EC_{50} > 0.092 mg/l (measured concentration, solubility limit) 48h EC_{50} >100 mg/L (nominal concentration)	Exposure: 48 h, static Measured and nominal concentration 20 ± 2 °C pH 7,24-7,63	Ziółkowska A., Wickiel G. (2013); Report No. 0003/0022/E
Growth inhibition test on algae OECD 201	*Pseudokirchneriella subcapitata*	Ferric pyrophosphate Batch 120327086	E_rLR_{50} >100 mg/L (nominal concentration) E_yLR_{50} >100 mg/L (nominal concentration) E_rLR_{50} ≥0,0212 mg/L (measured concentration) E_yLR_{50} ≥0,0212 mg/L (measured concentration)	Exposure: 72 h Measured and nominal concentration 23,5-23,8 °C pH 7.0-7.5	Heisterkamp I. (2015) Report No. 1040

[1] Indicate if the results are based on the measured or on the nominal concentration

Acute (short-term) toxicity to fish

The acute toxicity study of the test item, ferric pyrophosphate for rainbow trout (*Oncorhynchus mykiss*) was conducted according to OECD Guideline No 203. (Anonymous 13, 2013). The aim of the study was to determine LC_{50}, LC_0 and LC_{100} values calculated on the basis of observed fish mortality symptoms after 24, 48, 72 and 96 hours of exposure period following OECD 203. The iron content in a solution was determined by the inductively coupled plasma optical emission spectrometry (ICP-OES), based on a validated analytical method. Thereafter iron content was converted by the stoichiometry to the content of iron pyrophosphate $Fe_4(P_2O_7)_3$. The test material is non-toxic in the determined test item concentration 134 µg/L, being it's solubility limit, and corresponding to nominal concentration of 100 mg/L. During the experiment, neither mortality of fish was observed, nor signs of intoxication in any replicate of tested concentration being the limit concentration of the test item. Basing on the actual observations value 96h LC_{50} greater then 134 µg/L, which a limit of solubility item in the stock solution containing 100 mg of item in 1 L of medium, thus nominally greater then 100 mg/L. No acute aquatic toxicity recorded at levels up to the limit of water solubility

This study was already evaluated during Annex I inclusion of ferric pyrophosphate and it was accepted.

Acute (short-term) toxicity to aquatic invertebrates

The acute Daphnia sp. (*Daphnia magna*) immobilization test for test item, ferric pyrophosphate was conducted according to OECD Guideline No 202 (Ziółkowska A., Wickiel G., 2013). The aim of the study was to determine EC_{50}, EC_{20} and EC_{10} values calculated on the basis of observed immobilization after 24 and 48 hours of the exposure. The iron content in a solution was determined by the inductively coupled plasma optical emission spectrometry (ICP-OES), based on a validated analytical method. Thereafter iron content was converted by the stoichiometry to the content of iron pyrophosphate $Fe_4(P_2O_7)_3$. The test material is non-toxic in the determined test item concentration 92 µg/L, being it's solubility limit, and corresponding to nominal concentration of 100 mg/L. In the limit test neither immobilization of Daphnia, nor signs of intoxication in any replicate of tested concentration being the solubility limit concentration of the test item was observed. Basing on the actual observations value 48h EC_{50} > 92 µg/L being a solubility limit of test item in the stock solution containing 100 mg of test item in 1 L of ISO medium - no acute aquatic toxicity recorded at levels up to the limit of water solubility.

This study was already evaluated during Annex I inclusion of ferric pyrophosphate and it was accepted.

Acute (short-term) toxicity to algae or other aquatic plants

A growth inhibition test with *Pseudokirchneriella subcapitata* was conducted according to OECD 201 in order to investigate the effect of the test substance on the growth of algae. The test vessels were prepared in three replicates and the control vessels were prepared in six replicates. The specific growth rate, yield and their percent inhibition compared to the controls were calculated for each replicate after 72 hours. The algae test was performed with five nominal loading rates between 6.25 mg/L and 100 mg/L. Chemical analysis of the test item was based on measuring the iron content. The results of the iron analysis were control-corrected an the geometric mean of the corrected value was calculated and converted according to the stechiometry of ferric pyrophosphate. Exposure of *Pseudokirchneriella subcapitata* to ferric pyrophosphate at a nominal concentration of 100 mg/l (0.0212 mg/L measured) did not show any effects on growth rate or biomass over 72 hours. The E_rLR_{50} and E_yLR_{50} were calculated to be > 100 mg/L, the NOELR was ≥ 100 mg/L. Tested material – ferric pyrophosphate did not show ecotoxic effects within the range of given concentrations and parameters.

Acute (short-term) toxicity to other aquatic organisms

Based on the obtained study results and lack of toxic properties of ferric pyrophosphate towards aquatic organisms, further studies on aquatic organisms are considered unnecessary.

Long-term aquatic hazard

The chronic toxicity of ferric pyrophosphate studies to fish and daphnia were not conducted. But the studies for the BW01 GB formulation (plant protection product containing ferric pyrophosphate) are available.

In case of REACH Registration Dossier there were no aquatic toxicity studies conducted. Studies to determine the short-term and long-term toxicity of ferric pyrophosphate to fish, invertebrates and algae were not submitted. In accordance with Regulation (EC) No. 1907/2006 Annex XI, section 2 testing for a specific endpoint may be omitted if it is technically not possible to conduct the study as a consequence of the properties of the substance. Ferric pyrophosphate is an insoluble inorganic material and is not considered to be bioavailable in aquatic environments. This is demonstrated by the fact that iron is often added to effluents containing soluble phosphates in order to remove phosphorus (via making the phosphate insoluble) and prevent eutrophication in water bodies. As a result of the physicochemical properties, administration of precise and consistent does levels is not considered to be possible and as such aquatic testing is not considered to be technically possible.

Despite conducting short-term aquatic toxicity for ferric pyrophosphate, Applicant decided not to conduct long-term toxicity for active substance due to its physicochemical properties. However, for active substance

approval and plant protection product registration long-term aquatic toxicity study for formulation containing 3% of the ferric pyrophosphate were conducted. In such a case these studies can be used to obtain ecotoxicological endpoint acceptable for classification purpose. These tests represent a worst case scenario since the formulation which was tested contains chelating agent making Iron more bioavaliable in water solutions and thus potentially more toxic than it's non-soluble form.

The summary of the chronic aquatic toxicity studies evaluated during Annex I inclusion of ferric pyrophosphate is reported below. Only information considered adequate, reliable and relevant for the classification proposal has been included.

Table 22**: Summary of relevant information on** chronic **aquatic toxicity**

Method	Species	Test material	Results[1]	Remarks	Reference
Long-term and chronic toxicity to fish OECD 210	Zebrafish *Danio rerio*	BW01 GB Batch: 032014-P82 Content of active substance: 3% of iron pyrophosphate	**NOEC =0.138 mg a.s./L** NOEC=4.6 mg product/L - measured concentration (10 mg product/Lnom)	Exposure: 30-days 26.20 °C- 27.50°C pH 8.10-8.16	Anonymous 14 (2014) Report No. 0001/0109/E
Daphnia reproduction test OECD 211	*Daphnia magna*	BW01 GB Batch: 032014-P82 Content of active substance: 3% of iron pyrophosphate	**NOECreproduction =3 mg a.s./Lnom** NOECreproduction =100 mg product/Lnom (Concentrations were measured only for the lowest (6.4 mg/L) and the highest (250 mg/L) nominal test item concentrations. Mean measured concentrations of test item in medium was 4 mg/L for both - the lowest and the highest - nominal concentrations)	Exposure: 21-days, 20 ± 2 °C pH 7.4-8.01	Winkler J. (2014) Report No. 0001/0111/E
Growth inhibition test on algae OECD 201	*Pseudokirchneriella subcapitata*	Ferric pyrophosphate Batch 120327086	**NOELR ≥100 mg/L** (nominal concentration) NOELR ≥0.0212 mg/L (measured concentration)	Exposure: 72 h 23.5-23.8 °C pH 7.0-7.5	Heisterkamp I. (2015) Report No. 1040

[1] Indicate if the results are based on the measured or on the nominal concentration

Chronic toxicity to fish

Since ferric pyrophosphate is a substance virtually insoluble in water and the acute toxicity study in fish demonstrated a lack of ferric pyrophosphate toxicity within the limit of its solubility, the Fish Early Life Stage (FELS) test was conducted according to OECD TG 210 for the BW01 GB formulation (plant protection product containing ferric pyrophosphate).

The aim of the study was to determine the highest observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The content of the test item in the medium was rated indirectly by analysis of the iron content. The iron content was determined by ICP-OES technique. The content of product (formulation) in solutions were calculated based on the determined percentage content of iron in the product (formulation) and based on determined content of iron in solution of product (formulation). Based on the research and statistical calculations indicated that the test material BW01 GB at the concentration 4.6 mg product/L (corresponding to 0.138 mg a.s./L) (nominal concentration - 10 mg product/L) has no effect on the percentage hatching, the survival or growth of organisms (expressed as weight and length change). Technically, the OECD 210 Guideline (FELS) is not a 'chronic' test but a sub-chronic test on sensitive life stages. It is widely accepted as a predictor of chronic toxicity and is used as such for purposes of classification in the harmonised system.
The FELS test conducted for the representative formulation demonstrated that the material studied is not toxic to *Danio rerio* in the early developmental stages (Anonymous 14 2014).

Chronic toxicity to aquatic invertebrates

Since ferric pyrophosphate is a substance virtually insoluble in water and the acute toxicity study for *Daphnia magna* demonstrated a lack of ferric pyrophosphate toxicity within the limit of its solubility, a study of reproductive and developmental toxicity to *Daphnia magna* (according to OECD TG 211) was conducted for the representative formulation (plant protection product containing ferric pyrophosphate). The main aim of the study was to determine the influence of the test item on Daphnia's reproduction and growth. In addition the adults' mortality was evaluated, as well as the observation of the other negative effect of test item, like loss of the reproduction abilities. As habitat for animals and diluent for the preparation of tested solutions OECD 211 recommends medium M4 or M7. However, due to the content of Na2EDTA, they could not be used in the study, since this compound would create complexes with iron ion originating from the test item. It would make the determination of the concentration of iron in solution impossible. Therefore, as habitat for animals and diluent for the preparation of tested solutions ISO medium was used, which is one of the media recommended by the OECD 202. ISO medium composition is known. In addition, Daphnia culture in laboratory is carried out at medium ISO, and develops and reproduces properly. It was predicted that the validity criteria for the minimum number of produced at the end of the experiment offspring will be passed, what, according to the OECD 211 is a criterion allowing the use ofthe medium. Representative formulation demonstrates no toxic effects on *Daphnia magna* reproduction and development up to concentration 100 mg product/L (corresponding to 3 mg a.s/L) (J. Winkler 2014).

Chronic toxicity to algae or other aquatic plants

Please refer to previous point 11.5.3 where the toxicity tests with the substance on algae are included.

Chronic toxicity to other aquatic organisms

Based on the study results obtained for aquatic organisms and a lack of toxic properties of both ferric pyrophosphate and formulation containing ferric pyrophosphate, further studies on aquatic organisms are considered unnecessary.

Comparison with the CLP criteria

Acute aquatic hazard

Ferric pyrophosphate may be transformed by typical (simple) environmental processes to ferric trivalent ion Fe3+ and to pyrophosphate anion. Fe3+ is a vital substance (essential metal) in broad spectrum of organisms including aquatic ones. The aquatic toxicity was evaluated in a weigh of evidence approach with read across data.

For classification purposes, the toxicity value of calcium hydrogenothophosphate are considered for justification of the non-metallic ion PO_4^{3-}. These read across data reveal that no toxicity arises from the non-metallic ion PO_4^{3-} released form compound.

Ecotoxicological data for three trophic levels non-metallic ion PO_4^{3-} has been obtained from registration report for the iron (III) orthophosphate, available from the page:

https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13292/6/2/1

Table 23: Acute ecotoxicological data for $CaHPO_4$

Test substance	pH	Test organism	Test duration	Effect [mg /L]	Reference
FISH					
Short-term exposure					
$CaHPO_4$	7.18-7.97	*Oryzias latipes*	acute 96h	LC_{50} > 13.5$_{mm}$ LC_{50} > 100$_{nom}$	Kim et al. 2013
DAPHNIDS AND OTHER INVERTEBRATES					
Short-term exposure					
$CaHPO_4$	7.73-8.18	*Daphnia magna*	acute 48h	EC_{50} > 2.75$_{mm}$ EC50 > 100$_{nom}$	Kim et al. 2013
AQUATIC ALGAE					
Short-term exposure					
$CaHPO_4$	Control: 9.06 - 8.36 0.3 mg/L: 8.83 - 8.39 1.0 mg/L: 8.84 - 8.37 3.1 mg/L: 8.87 - 8.35 9.8 mg/L: 8.89 - 8.30 31.3 mg/L: 8.79 - 8.32 100.0 mg/L: 8.57 - 8.44	*Pseudokirchneriella subcapitata*	acute 72h	ErC_{50}> 4.4$_{m}$ ErC_{50} > 100$_{nom}$	Kim et al. 2013

nom – nominal test substance concentrations
m -measured test concentrations
mm - mean measured concentration

For classification purposes, the ecotoxicological reliable data (LC_{50}/EC_{50} for acute toxicity of dissolved iron compound $FeCl_3$) has been taken into account for justification of the metallic ion. Fe^{3+}. $FeCl_3$ is a water-soluble iron salt which makes using it's data a worst case scenario in reference to almost insoluble ferric pyrophosphate.

Ecotoxicological data for three trophic levels has been obtained from registration report for the $FeCl_3$, available from the page:

https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/16109/6/2/7

According to the information provided on the ECHA dissemination site, for the purpose of classification of $FeCl_3$, selected acute ecotoxity data on Fe for fish and daphnia are from the EURAS critical review (Vangheluwe & Versonnen 2004), taking into account the studies results with soluble form of iron compounds.

Table 24: Acute ecotoxicological data for Fe^{3+} ion

Test substance	pH	Test organism	Test duration	Effect [mg Fe/L]	Reference
FISH					
Short-term exposure					
$FeCl3.6H_2O$	6.3	*Lepomis macrochirus*	acute 96h	LC_{50} = 20.3	Birge et al. 1985
$FeCl3.6H_2O$	6.7	*Pimephales promelas*	acute 96h	LC_{50} = 21.8	Birge et al. 1985
$FeSO_4.6H_2O$	7.35	*Oncorhynchus mykiss*	acute 96h	LC_{50} = 16.6	Mattock 2002
DAPHNIDS AND OTHER INVERTEBRATES					
Short-term exposure					
$FeCl3.6H_2O$	6.1	*Daphnia pulex*	acute 48h	EC_{50} = 12.9	Birge et al. 1985
$FeCl3.6H_2O$	7.7	*Daphnia magna*	acute 48h	EC_{50} = 9.6	Biesinger & Christensen 1972
$FeSO_4.7H_2O$	6.25	*Daphnia magna*	acute 48h	EC_{50} = 1.29	LISEC study no. WE-01-225. Draft
$FeSO_4$	7.6	*Daphnia magna*	acute 24h	EC_{50} = 5.25	Lilius et al. 1995
$FeSO_4$	7.6	*Daphnia pulex*	acute 24h	EC_{50} = 36.9	Lilius et al. 1995
$FeSO_4$	n.r.	*Daphnia magna*	acute 24h	EC_{50} = 17	Calleja et al. 1994
$FeSO_4$	n.r.	*Brachionus calyciflorus*	acute 24h	EC_{50} = 12	Calleja et al. 1994
AQUATIC PLANTS					
Short-term exposure					
FeCl3	7.5	*Lemna minor*	acute 4 days	EC_{50}=3.7	Wang 1986

- Birge WJ, Black JA, Westerman AG, Short TM, Taylor SB, Bruser DM, Wallingford ED (1985). Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warmwater species of aquatic life in the Commonwealth of Kentucky. Memorandum of Agreement No. 5429, Kentucky Natural Resources and Environmental Protection Cabinet.
- Biesinger KE, Christensen GM (1972). Effects of various metals on survival, growth, reproduction and metabolism of Daphnia magna. Journal of Fisheries Research Board of Canada 29: 1691-1700.
- Wang W. 1986. Toxicity tests of aquatic pollutants by using common duckweed. DOI 10.1016/0143-148X(86)90028-5 Environmental Pollution Series B 11(1):1-14.

Acute ERVcompound = acute ERV of the metal compound = acute ERV of metal ion x (Molecular weight of metal compound /atomic weight of the metal).

To reflect the stoichiometry of the compound, the molecular weight of Fe has been multiplied by four (According to the note in the Guidance)

The range of LC_{50}/EC_{50} values is 1.29 (around pH 6) – 36.9 (around pH 8) mg Fe/L. Taking into account values presented above the values of ERVcompound acute can be calculate.

Acute ERVferric pyrophosphate = 3.7 x (745.21/223.36) = 12.34 (around pH 8)

Acute ERVferric pyrophosphate = 16.6 x (745.21/223.36) = 55.38 (around pH 7)

Acute ERVferric pyrophosphate = 1.29 x (745.21/223.36) = 4.30 (around pH 6)

Solubility of ferric pyrophosphate

Accordin to the Guidance on the Application of CLP Criteria Version 5.0 July 2017: „*Metal compounds that have lower water solubility than the acute ERV through a 24-hour Dissolution Screening test or estimated from the solubility product, are considered as poorly.*"

Ferric pyrophosphate data from 24-hour Dissolution Screening test is not available. Therefore the solubility was assessed based on the read-across data for iron ortophosphate, Iron ortophosphate is a ferric phosphate salt, composed of a phosphate as anion ($PO4^{3-}$) and iron as cation (Fe^{3+}). Generally the water solubility of phosphates appears to be related to the inorganic cation. Taking into account the similar structure, physical-chemical properties, environmental fate properties and ecotoxicological profile of substances, iron orthophosphate data can be used to assess the water solubility. The results of the 24-hours Dissolution Screening test have been obtained from REACH registration dossier for the iron ortophosphate.

Table 25. Results of the 24-hours Dissolution Screening test for the Iron ortophosphate

DATA ELEMENTS	VALUE	Test method
Screening test (24 h) at 100 mg/L loading	pH 6: 11.23 µg/L	OECD 29

In REACH registration dossier the robust study summary is provided. The key study (Klawonn T. (2016)) to determine the transformation/dissolution of the test items iron(lll)orthophosphate anhydrous (CAS 10045 -86 -0) and iron (III) orthophosphate dihydrate (CAS 14567 -75 -0) was conducted according to the OECD guidance document 29 (2001) and GLP. The test was performed with both test items at pH 6 and 8 to cover acidic as well as basic conditions in environment. As requested, the test was conducted with a loading of 100 mg/L of both test items over 24 hours and one sampling after one day. Solution pH, oxygen concentrations and total dissolved iron concentrations were measured at each sampling time. lron(lll)orthophosphate at pH 6 exhibited the highest dissolved Fe concentration in the screening after 24 h with 11.229 ± 4.544 µg Fe/L. The mean dissolved amount of Fe after 168 h of testing at pH 6 with a loading of 100 mg/L was 21.062 ± 9.214 µg Fe/L. This corresponds to a calculated solubility of 58.506 ± 25.594 µg test item/L. The mean dissolved amount of Fe after 168 h of testing at pH 6 with a loading of 10 mg/L was 0.884 ± 0.242 µg Fe/L. This corresponds to a calculated solubility of 2.456 ± 0.672 µg test item/L. At the loadings of 10 and 100 mg test item/L the dissolved Fe concentrations decreases over time. This is probably due to formation of hydroxides and subsequent precipitation.

Transformation Dissolution screening outcome:

The substance fail the 24 h screening Transformation Dissolution test given the dissolution at a loading of 100 mg/l :

- at pH 6 is 11.23 µg/L < acute ERV of the soluble ion being 4.3 mg/L (around pH 6)

The test result at pH 8 was not reported in REACH registration dossier. However, it was emphasized that the highest dissolved Fe concentration in the screening test was determined at pH 6. This clearly show that solubility at pH 8 is lower than 11.23 µg/L. Therefore, it can be concluded that acute ERV of the soluble ion being 12.34 mg/L (around at pH 8) is much higher than the solubility at pH 8.

Conclusion: Ferric pyrophosphate is considerd as **poorly soluble metal compound.**

According to the Guidance on the Application of CLP Criteria Version 5.0 July 2017: „*Where the acute ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as acute ERVcompound) is greater than 1 mg/L, the metal compounds need not to be considered further in the classification scheme for acute hazard*."

Taking into account values presented above, values of acute ERVcompounds can be calculated. The range of acute ERV ferric pyrophosphate is 4.30 – 55.38. In case of the lowest value of EC_{50} (1.29 mg Fe/L) taken as acute ERV for classification purpose, calculated acute ERV ferric pyrophosphate value is 4.30. This value is greater than 1 mg/L therefore, the metal compound need not be considered further in classification scheme and it is not classified as acute term hazard.

Conclusion: The lowest acute ERV at 4.3 mg/L is greater than 1 mg/L, therefore there is no aquatic acute classification of Ferric pyrophosphate proposed.

Long-term aquatic hazard (including bioaccumulation potential and degradation)

For classification purposes the ecotoxicological data (NOEC and EC_{50} for long-term toxicity of dissolved iron compound $FeCl_3$) has been taken into account. $FeCl_3$ is a water soluble iron salt which makes using it's data a worst case scenario in reference to almost insoluble Ferric Pyrophosphate.

Ecotoxicological data for three trophic levels were obtained from registration report for the $FeCl_3$ available from the following pages:

https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/16109/6/2/7

According to the information provided on the ECHA dissemination site, for the purpose of classification of $FeCl_3$, selected chronic ecotoxity data on Fe for fish and daphnia are from the EURAS critical review (Vangheluwe & Versonnen 2004), taking into account the studies results with soluble form of iron compounds.

Table 24: Long term ecotoxicological data for $FeCl_3$

Test substance	pH	Test organism	Test duration	Effect [mg Fe/L]	Reference
FISH					
Long-term exposure					
FeCl3	7.7	*Pimephales promelas*	chronic 33d	NOEC = 1.00	Birge et al. 1985
DAPHNIDS AND OTHER INVERTEBRATES					
Long-term exposure					
FeCl3	7.6	*Daphnia pulex*	chronic 21d	NOEC = 0.63	Birge et al. 1985
FeCl3	7.7	*Daphnia magna*	chronic 21d		Biesinger & Christensen 1972

				EC_{50} = 5.2	
Long-term exposure					
FeCl3	7.5`	*Spirodela polyrhiza*	Chronic 14 days	NOEC<0.56	Sinha et al 1994

- Birge WJ, Black JA, Westerman AG, Short TM, Taylor SB, Bruser DM, Wallingford ED (1985). Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warmwater species of aquatic life in the Commonwealth of Kentucky. Memorandum of Agreement No. 5429, Kentucky Natural Resources and Environmental Protection Cabinet.
- Biesinger KE, Christensen GM (1972). Effects of various metals on survival, growth, reproduction and metabolism of Daphnia magna. Journal of Fisheries Research Board of Canada 29: 1691-1700.
- Sinha S, Rai UN, Chandra P (1994). Accumulation and toxicity of iron and manganese in Spirodela polyrrhiza (L.) schieiden. Bulletin of Environmental Contamination and Toxicology. 53(4):610–7.

Chronic $ERV_{\mathbf{compound}}$ = chronic ERV of the metal compound = chronic ERV of metal ion x (Molecular weight of metal compound /atomic weight of the metal).

To reflect the stoichiometry of the compound, the molecular weight of Fe has been multiplied by four. (According to the note in the Guidance)

The range of chronic NOEC or EC_{50} (from 21d chronic study on *Daphnia magna*) is 0.56 – 5.2 mg Fe/L.

Chronic $ERV_{\mathbf{ferric\ pyrophosphate}}$ = 0.56 x (745.21/223.36) = 1.87

Chronic $ERV_{\mathbf{ferric\ pyrophosphate}}$ = 5.2 x (745.21/223.36) = 17.35

According to the Guidance on the Application of CLP Criteria Version 5.0 July 2017: "*Where the chronic ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as chronic ERVcompound) is greater than 1 mg/L, the metal compounds need not to be considered further in the classification scheme for long-term hazard*."

Taking into account values presented above, values of chronic $ERV_{compounds}$ can be calculated. The range of chronic $ERV_{\text{ferric pyrophosphate}}$ is 1.87 – 17.35. In case of the lowest value of NOEC (0.56 mg Fe/L) taken as chronic ERV for classification purpose, calculated chronic $ERV_{\text{ferric pyrophosphate}}$ value is 1.87. This value is greater than 1 mg/L therefore, the metal compound need not be considered further in classification scheme and it is not classified as long term hazard.

Conclusion: The lowest chronic ERV at 1.87 mg/L is greater than 1 mg/L, therefore there is no aquatic long-term classification of Ferric pyrophosphate proposed.

CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Read across analysis does not allow to classify the ferric pyrophosphate as hazardous for aquatic environment according to CLP Regulation.

Ferric pyrophosphate is considerd as poorly soluble metal compound.

The acute $ERV_{\text{ferric pyrophosphate}}$ value is 4.30 mg/L. It is greater than 1 mg/L. This value is significaly greater than solubility of this substance

The chronic $ERV_{\text{ferric pyrophosphate}}$ value is 1.87 mg/L. It is greater than 1 mg/L. This value is significaly greater than solubility of this substance.

In result, the ferric pyrophosphate need not to be considered in the classification scheme for acute and chronic hazards.

According to CLP-Regulation no classification with regard to the environment is required.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Ferric pyrophosphate is considered as insoluble metal compound. The DS proposed for ferric pyrophosphate no classification for acute aquatic toxicity and no classification for chronic aquatic toxicity. The conclusion was based on calculated acute ERV value of 4.30 mg/L and calculated chronic ERV value of 1.87 mg/L.

Degradation

Ferric pyrophosphate is an inorganic substance. Since the substance is inorganic the biodegradation concept does not apply.

Environmental transformation of metals or inorganic metals compounds

The DS presented ferric pyrophosphate as a stable non-volatile inorganic salt, virtually insoluble in water. Iron, the main component of ferric pyrophosphate, is a chemical element which composes about 5% of the Earth's crust and is found in the form of minerals such as: hematite, magnetite, siderite or pyrite. Generally, iron is found in two oxidation states - reduced, as ferrous ion Fe^{2+}, or oxidized, as ferric ion Fe^{3+}. Fe^{2+} is more physiologically important for plants, however Fe^{3+} is more stable and represents the main ion distributed in the environment. Iron is an essential element for plants and its availability for plants is increased by various mechanism.

Phosphorus, the other main component of ferric pyrophosphate, is also an essential element important for the functioning of every cell. Phosphorus compounds in soil display great diversity both in terms of chemical forms and the strength of bonding with the solid phase. The DS presented extended discussion on iron and phosphorus behaviour in soils and fertilizers as sources for both elements.

Data from transformation/dissolution test for ferric pyrophosphate according to the OECD TG 29 is not available. The DS proposed the analysis of transformation/dissolution to be based on the read-across data for iron orthophosphate, taking into account the similar structure, physical-chemical properties, environmental fate properties and ecotoxicological profile of the substances. The study to determine the transformation/dissolution of the test items iron(III)orthophosphate anhydrous (CAS 10045-86-0) and iron (III) orthophosphate dihydrate (CAS 14567-75-0) was conducted according to the OECD TG 29 (2001) and GLP, at both pH 6 and 8 to cover acidic as well as basic conditions in environment. As requested, the test was conducted with a loading of 100 mg/L of both test items over 24 hours and one sampling after one day. The maximum amount of iron in the screening test was quantified at pH 6 applying iron(III)orthophosphate (CAS 10045-86-0 [anhydrous]). Therefore, the full test had been subsequently conducted with iron(III)orthophosphate (CAS 10045-86-0) at pH 6. Solution pH, oxygen concentrations and total dissolved iron concentrations were measured at each sampling time. lron(III)orthophosphate (CAS 10045-86-0 [anhydrous]) at pH 6 exhibited the highest dissolved Fe concentration in the screening after 24 h with 11.229 ± 4.544 µg Fe/L. The mean dissolved amount of Fe after 168 h of testing at pH 6 with a loading of 100 mg/L was 21.062 ± 9.214 µg Fe/L. This corresponds to a

calculated solubility of 58.506 ± 25.594 µg test item/L. At the loadings of 10 and 100 mg test item/L the dissolved Fe concentrations decreases over time. This is probably due to formation of hydroxides and subsequent precipitation.

Bioaccumulation

The DS concluded that since ferric pyrophosphate is insoluble in water, octanol/water partition coefficient cannot be established. Essentiality of both elements iron and phosphorus ruled out bioconcentration of ferric pyrophosphate in organisms. The DS concluded there is a low potential for bioaccumulation of ferric pyrophosphate in aquatic organism.

Acute aquatic hazard

The summary of the acute aquatic toxicity studies of ferric pyrophosphate is reported in Table below. Only information considered adequate, reliable and relevant for the classification proposal has been included.

***Table**: Summary of relevant information on acute aquatic toxicity*

Method	Species	Test material	Results	Remarks	Reference
Acute toxicity to rainbow trout OECD TG 203	rainbow trout (*Oncorhynchus mykiss*)	Ferric pyrophosphate Batch 120327086	LC_{50} > 0.134 mg/L (measured concentration; solubility limit) – LC_{50} > 100 mg/L (nominal concentration)	Exposure: 96h, static Measured and nominal concentration 14,40°C - 16,10°C pH 8,5	Anonymous 13, 2013; Report No. 0003/0024/E
Aquatic invertebrates short-term toxicity OECD TG 202	*Daphnia magna*	Ferric pyrophosphate Batch 120327086	48h EC_{50} > 0.092 mg/L (measured concentration, solubility limit) 48h EC_{50} >100 mg/L (nominal concentration)	Exposure: 48h, static Measured and nominal concentration 20 ± 2°C pH 7.24-7.63	Ziółkowska, Wickiel, 2013; Report No. 0003/0022/E
Growth inhibition test on algae OECD TG 201	*Pseudokirchneriella subcapitata*	Ferric pyrophosphate Batch 120327086	E_rLR_{50} ≥ 0.0212 mg/L (measured concentration) E_yLR_{50} ≥ 0.0212 mg/L (measured concentration) E_rLR_{50} > 100 mg/L (nominal concentration) E_yLR_{50} > 100 mg/L (nominal concentration)	Exposure: 72 h Measured and nominal concentration 23.5-23.8°C pH 7.0-7.5	Heisterkamp, 2015; Report No. 1040

Acute (short-term) toxicity to fish

The acute toxicity of ferric pyrophosphate toward rainbow trout (*Oncorhynchus mykiss*) was studied, according to OECD TG 203 (Anonymous 13, 2013), for an exposure period of 96 hours. The iron content in the test solution was measured by the inductively coupled plasma optical emission spectrometry (ICP-OES). The ferric pyrophosphate was found non-toxic at

concentration of 134 µg/L, being its solubility limit, corresponding to nominal concentration of 100 mg/L. The 96h LC_{50} greater than 134 µg/L was defined.

Acute (short-term) toxicity to aquatic invertebrates

The acute toxicity of ferric pyrophosphate toward Daphnia sp. (*Daphnia magna*) was studied in an immobilization test, conducted according to OECD TG 202, for an exposure period of 48 hours (Ziółkowska and Wickiel, 2013). The iron content in the test solution was measured by the inductively coupled plasma optical emission spectrometry (ICP-OES). The ferric pyrophosphate was non-toxic at a concentration of 92 µg/L, being its solubility limit, and corresponding to nominal concentration of 100 mg/L. The 48h EC_{50} greater than 92 µg/L was defined.

Acute (short-term) toxicity to algae or other aquatic plants

A growth inhibition test with Pseudokirchneriella subcapitata was conducted according to OECD TG 201 (Heisterkamp, 2015). The test vessels were prepared in three replicates and the control vessels were prepared in six replicates with five nominal loading rates (LR) between 6.25 mg/L and 100 mg/L. The specific growth rate, yield and their percent inhibition compared to the controls were calculated for each replicate after 72 hours based on iron concentration measurement. Exposure of Pseudokirchneriella subcapitata to ferric pyrophosphate at a nominal concentration of 100 mg/L (0.0212 mg/L measured) did not show any significant effects on growth rate or biomass over 72 hours. The ErLR50 and EyLR50 were calculated to be > 100 mg/L, the NOELR was ≥ 100 mg/L.

Acute aquatic hazard

Ferric pyrophosphate hydrolyses in aqueous solution releasing Fe^{3+} ions and pyrophosphate ions which further dissociate to orthophosphate ions.

For classification purposes, the DS considered the toxicity values of calcium hydrogenorthophosphate for justification of the toxicity of the non-metallic ion PO43-.

Ecotoxicological data for three trophic levels are available for non-metallic ion PO43-, obtained from registration report for the iron (III) orthophosphate, available at the following link:

https://echa.europa.eu/da/registration-dossier/-/registered-dossier/13292/6/2/1

Table: *Acute ecotoxicological data for $CaHPO_4$*

Test substance	pH	Test organism	Test duration	Effect [mg/L]	Reference
FISH					
$CaHPO_4$	7.18-7.97	*Oryzias latipes*	acute 96h	$LC_{50} > 13.5_{mm}$ $LC_{50} > 100_{nom}$	Kim *et al.*, 2013
DAPHNIDS AND OTHER INVERTEBRATES					
$CaHPO_4$	7.73-8.18	*Daphnia magna*	acute 48h	$EC_{50} > 2.75_{mm}$ $EC50 > 100_{nom}$	Kim *et al.*, 2013
AQUATIC ALGAE					
$CaHPO_4$	Control: 9.06 - 8.36 0.3 mg/L: 8.83 - 8.39 1.0 mg/L: 8.84 - 8.37 3.1 mg/L: 8.87 - 8.35 9.8 mg/L: 8.89 - 8.30 31.3 mg/L: 8.79 - 8.32 100.0 mg/L: 8.57 - 8.44	*Pseudokirchneriella subcapitata*	acute 72h	$ErC_{50} > 4.4_{m}$ $ErC_{50} > 100_{nom}$	Kim *et al.*, 2013

nom – nominal test substance concentrations

m -measured test concentrations
mm - mean measured concentration

For the classification of Fe^{3+}, reliable data (LC_{50}/EC_{50} for acute toxicity of dissolved iron compound $FeCl_3$) has been taken into account from the EURAS critical review (Vangheluwe & Versonnen, 2004), obtained with $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$, available at the following link:

https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/16109/6/2/7

***Table**: Acute ecotoxicological data for Fe^{3+} ion from the EURAS critical review (Vangheluwe & Versonnen, 2004)*

Test substance	Test Conditions	Test organism	Test duration	Endpoint Nominal/ Measured	Effect [mg Fe/L]	Reference
FISH						
$FeCl_3 \cdot 6H_2O$	pH: 6.3; T: 22; H: 100; Alk: 24 Test medium: Reconstituted ASTM water	*Lepomis macrochirus*	96h	Survival total Fe measured	LC_{50} = 20.3	Birge *et al.*, 1985
$FeCl_3 \cdot 6H_2O$	pH: 6.7; T: 22; H: 100; Alk: 30 Test medium: Reconstituted ASTM water	*Pimephales promelas*	96h	Survival total Fe measured	LC_{50} = 21.8	Birge *et al.*, 1985
$FeSO_4 \cdot 6H_2O$	pH: 6.0-7.1; H: 56-60; Alk: 32, a Test medium: Dechlorinated / carbon filtered tap water	*Oncorhynchus mykiss*	96h	Survival total dissolved Fe, measured, filtered 0.2 µm filter	LC_{50} = 16.6	Mattock, 2002a
$FeSO_4 \cdot 6H_2O$	pH: 6.9-7.0; T: 13-15; H: 64-97 Test medium: Dechlorinated / carbon filtered tap water	*Oncorhynchus mykiss*	96h	Survival total dissolved Fe, measured, c	LC_{50} > 27.9	Mattock, 2002b
$FeSO_4$	pH: 5.5 pH: 6 pH: 7 Test medium: Carbon filtered river water	*Salvelinus fontinalis*	96h	Survival total and total dissolved Fe, measured	pH 5.5 LC_{50} = 0.41 pH 6 LC_{50} = 0.48 pH 7 LC_{50} = 1.75	Decker & Menendez, 1974
$FeSO_4$	pH: 7.1; small carp pH 7.1; large carp Test medium: not reported	*Cyprinus carpio*	96h	Survival nominal	LC_{50} = 0.83 LC_{50} = 1.62	Alam & Maugham, 1992
$FeSO_4 \cdot 6H_2O$	pH: 5; T: 25; H:40 pH: 7; T: 25; H: 40 pH: 9; T: 25; H: 40 Test medium: Aerated, aged tap water	*Danio rerio*	48h	Survival nominal	LOEC > 32 LOEC > 32 LOEC > 32	Dave, 1985
DAPHNIDS AND OTHER INVERTEBRATES						
$FeCl_3 \cdot 6H_2O$	pH: 6.1; T: 20; H: 96; Alk: 28 Test medium: Reconstituted ASTM water	*Daphnia pulex*	48h	Immobility measured	EC_{50} = 12.9	Birge *et al.*, 1985
$FeCl_3 \cdot 6H_2O$	pH: 7.7; T: 18; (room T); static Test medium: Lake Superior water	*Daphnia magna*	48h	Immobility total Fe measured	EC_{50} = 9.6	Biesinger & Christensen, 1972
$FeSO_4 \cdot 7H_2O$	pH: 6.0; T: 21.6-22 Test medium: Reconstituted water	*Daphnia magna*	48h	Immobility total dissolved Fe, measured	EC_{50} = 1.29	LISEC study no. WE-01-225. Draft
$FeSO_4$	pH: 7.6 Test medium: Standard reference water	*Daphnia magna*	24h	Immobility nominal	EC_{50} = 5.25	Lilius *et al.*, 1995
$FeSO_4$	pH: 7.6 Test medium: Standard reference water	*Daphnia pulex*	24h	Immobility nominal	EC_{50} = 36.9	Lilius *et al.*, 1995

$FeSO_4$	SOP Test medium: Reconstituted water	*Daphnia magna*	24h	Immobility nominal	EC_{50} = 17	Calleja *et al.*, 1994
$FeSO_4$	SOP Test medium: ASTM E1440-91	*Brachionus calycifloru, Rotifer*	24h	Survival nominal	LC_{50} = 12	Calleja *et al.*, 1994
$FeSO_4 \cdot 7H_2O$	pH: 7.6; T: 13; H: 240; Alk: 400 Test Medium: Filtered, aerated tubewell water	*Daphnia magna*	48h	Immobility nominal	EC_{50} = 7.2	Khangarot & Ray, 1989
$FeCl_3$	pH: 8.2-8.4 Test medium: Lake Eria water	*Daphnia magna*	64h	Immobility nominal	'threshold' < 6.1	Anderson, 1950
AQUATIC PLANTS						
Fe^{3+}	pH: 7.5, T: 27; Test medium: deionized water	*Lemna minor*	4 days	growth nominal	EC_{50} = 3.7	Wang, 1986

Finally, the DS calculated for each pH range the acute $ERV_{compound}$ taking into account molecular weights of iron salts and compound stoichiometry:

Acute $ERV_{ferric\ pyrophosphate}$ = 3.7 x (745.21/223.36) = 12.34 mg/L (around pH 8)

Acute $ERV_{ferric\ pyrophosphate}$ = 16.6 x (745.21/223.36) = 55.38 mg/L (around pH 7)

Acute $ERV_{ferric\ pyrophosphate}$ = 1.29 x (745.21/223.36) = 4.30 mg/L (around pH 6)

The DS considered as a next step solubility of ferric pyrophosphate, assessed based on the read-across data for iron orthophosphate.

The results of the 24 hours Dissolution Screening test for the iron orthophosphate have been obtained from REACH registration dossier.

DS concluded that highest dissolution value at pH 6, 11.23 µg/L < acute ERV of the soluble ion being 4.3 mg/L (at pH 6) thus confirming that ferric pyrophosphate is an insoluble metal compound.

The dissolution of orthophosphate at pH 8 was not reported in REACH registration dossier, however, it was emphasized that the highest dissolved Fe concentration in the screening test was determined at pH 6. This clearly showed that solubility at pH 8 was lower than 11.23 µg/L. Therefore, it can be concluded that acute ERV of the soluble ion being 12.34 mg/L (around at pH 8) is much higher than the solubility at pH 8.

Long-term aquatic hazard

Chronic toxicity studies with ferric pyrophosphate toward fish species and *Daphnia* were not available. The chronic toxicity studies toward Zebrafish *Danio rerio* and *Daphnia magna* were conducted with plant protection product containing 3% ferric pyrophosphate (BW01 GB formulation).

The DS noted that these studies can be used to obtain ecotoxicological endpoint acceptable for classification purposes.

The summary of the chronic aquatic toxicity studies is reported in Table below. Only information considered adequate, reliable and relevant for the classification proposal has been included.

***Table**: Summary of relevant information on chronic aquatic toxicity*

Method	Species	Test material	Results	Remarks	Reference
Long-term and chronic toxicity to fish OECD TG 210	Zebrafish *Danio rerio*	BW01 GB Batch: 032014-P82 Content of active substance: 3% of iron pyrophosphate	**NOEC = 0.138 mg a.s./L** NOEC = 4.6 mg product/L - measured concentration (10 mg product/L nom)	Exposure: 30-days 26.20°C- 27.50°C pH 8.10-8.16	Anonymous 14, 2014; Report No. 0001/0109/E
Daphnia reproduction test OECD TG 211	*Daphnia magna*	BW01 GB Batch: 032014-P82 Content of active substance: 3% of iron pyrophosphate	**$NOEC_{reproduction}$ = 3 mg a.s./L nom** $NOEC_{reproduction}$ = 100 mg product/L nom (Concentrations were measured only for the lowest (6.4 mg/L) and the highest (250 mg/L) nominal test item concentrations. Mean measured concentrations of test item in medium was 4 mg/L for both - the lowest and the highest - nominal concentrations)	Exposure: 21-days, 20 ± 2 °C pH 7.4-8.01	Winkler, 2014; Report No. 0001/0111/E
Growth inhibition test on algae OECD TG 201	*Pseudokirchneriella subcapitata*	Ferric pyrophosphate Batch 120327086	**$NOEL_R \geq 100$ mg/L** (nominal concentration) $NOEL_R \geq 0.0212$ mg/L (measured concentration)	Exposure: 72 h 23.5-23.8 °C pH 7.0-7.5	Heisterkamp, 2015; Report No. 1040

Chronic toxicity to fish

The Fish Early Life Stage test was conducted with Zebrafish (*Danio rerio*) according to OECD TG 210 using the BW01 GB formulation (plant protection product containing ferric pyrophosphate) as test item (Anonymous 14, 2014). The iron content during the test was determined by ICP-OES. Obtained results indicated that test material BW01 GB at the concentration of 4.6 mg product/L (corresponding to 0.138 mg a.s./L) has no effect on the percentage hatching, the survival or growth of organisms (expressed as weight and length change).

Chronic toxicity to aquatic invertebrates

Daphnia magna reproduction test was conducted according to OECD TG 211 with BW01 GB formulation (plant protection product containing ferric pyrophosphate) (Winkler, 2014). The recommended test medium M4 or M7 contains Na_2EDTA and was replaced in this case with ISO medium, recommended by the OECD TG 202. The validity criteria for the minimum number of produced offspring at the end of the experiment was passed and results accepted as valid for OECD TG 211. Obtained results demonstrate no toxic effects on *Daphnia magna* reproduction

and development up to concentration 100 mg product/L (corresponding to 3 mg a.s./L).

Chronic toxicity to algae or other aquatic plants

Please refer to data presented in section on acute aquatic toxicity where the toxicity tests with the substance on algae are included.

The DS used the ecotoxicological data (NOEC and EC_{50}) for long-term toxicity of dissolved iron compound $FeCl_3$ to read across for chronic toxicity data.

Ecotoxicological data for three trophic levels were obtained from the EURAS critical review (Vangheluwe & Versonnen, 2004) for the $FeCl_3$ available at the following link:

https://echa.europa.eu/pl/registration-dossier/-/registered-dossier/16109/6/2/7

***Table**: Long term ecotoxicological data for FeCl3 from the EURAS critical review (Vangheluwe & Versonnen, 2004)*

Test substance	Test conditions	Test organism	Test duration	Nominal/Measured	Endpoints	Effect [mg Fe/L]	Reference
FISH							
$FeCl_3$	pH: 7.7; TºC: 25; Hardness: 103; Alkalinity: 56 Test medium: reconstituted ASTM water	*Pimephales promelas*	33d	total Fe measured; total dissolved Fe, measured; total Fe(II) ion measured	Length	**NOEC = 1.00**	Birge et al. 1985
					Weight	**NOEC = 1.61**	
$Fe(OH)_3$	pH: 8.1; TºC: 11; Hardness: 159-180 Test medium:	*Oncorhynchus kisutch*	30d	total Fe measured; total dissolved Fe, measured; total Fe(II) ion measured	Survival	NOEC = 2.81	Smith & Sykora 1976
$FeSO_4.7H_2O$	pH: 7.7-7.9; TºC: 15.7 – 22.6	*Cyprinus carpio*	2 weeks	total Fe measured	Cortisol level	NOEC = 0.52	van Anholt *et al.*, 2002
DAPHNIDS AND OTHER INVERTEBRATES							
$FeCl_3$	pH: 7.6; TºC: 20; Hardness: 94; Alkalinity: 48 Test medium: Reconstituted ASTM water	*Daphnia pulex*	chronic 21d	total Fe measured; total dissolved Fe, measured; total Fe(II) ion measured	Immobility	NOEC = 2.51	Birge *et al.*, 1985
					Total offspring	**NOEC = 0.63**	
					Brood size	NOEC = 0.63	
					Aborted eggs	NOEC = 1.26	
					Length	NOEC = 1.26	
$FeCl_3 \cdot 6H_2O$	pH: 7.7; TºC: 18 (room TºC); static renewal Test medium: Lake Superior water	*Daphnia magna*	chronic 3 weeks	total Fe measured	Immobility, reproduction	$EC_{50\ immobility}$ = 5.9	Biesinger & Christensen, 1972
						$EC_{50\ reproduction}$= 5.2	
						EC_{16} reproduction= 4.4	
$FeSO_4 \cdot 7H_2O$	pH: 7.7-7.9; TºC: 15.7-22.6 Test medium: River water	*Daphnia magna*	2 weeks	total Fe measured	Reproduction	NOEC = 0.52	Van Anholt *et al.*, 2002
MACROPHYTES							
$FeCl_3$	pH: 7.5	*Spirodela polyrhiza*	Chronic 14 days	Measured total iron	Growth effects	NOEC < 0.56	Sinha *et al.*, 1994 Long-term aquatic toxicity data on Macrophyte from the OECD (2007)

Finally, the DS calculated for each pH range chronic $ERV_{compound}$ taking into account molecular weights of

iron salts and compound stoichiometry:

Chronic $ERV_{\text{ferric pyrophosphate}}$ = 0.56 x (745.21/223.36) = 1.87 mg/L

Chronic $ERV_{\text{ferric pyrophosphate}}$ = 5.2 x (745.21/223.36) = 17.35 mg/L

Comparison with the CLP criteria

Following the CLP guidance: *„Where the acute ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as acute $ERV_{compound}$) is greater than 1 mg/L, the metal compounds need not to be considered further in the classification scheme for acute hazard*."

The DS, based on calculated acute $ERV_{\text{ferric pyrophosphate}}$ values, concluded that lowest one (4.3 mg/L) is above 1 mg/L and warrants no classification for ferric phosphate for acute aquatic hazard.

Following the CLP guidance: "*Where the chronic ERV for the metal ions of concern corrected for the molecular weight of the compound (further called as chronic $ERV_{compound}$) is greater than 1 mg/L, the metal compounds need not to be considered further in the classification scheme for long-term hazard*."

The DS, based on calculated chronic $ERV_{\text{ferric pyrophosphate}}$ values, concluded that lowest one (1.87 mg/L) is above 1 mg/L and warrants no classification for ferric phosphate for chronic aquatic hazard.

Conclusion on classification and labelling for environmental hazards

The DS concluded on the classification of ferric pyrophosphate for environmental aquatic hazard according to CLP Regulation.

Ferric pyrophosphate is considered an insoluble metal compound.

The calculated acute $ERV_{\text{ferric pyrophosphate}}$ value of 4.30 mg/L is greater than 1 mg/L.

The calculated chronic $ERV_{\text{ferric pyrophosphate}}$ value is 1.87 mg/L is greater than 1 mg/L.

According to CLP-Regulation both values warrant no classification for ferric phosphate for aquatic hazards.

Comments received during consultation

One comment was received from one MSCA demonstrating agreement with the proposed approach and outcome for ferric pyrophosphate classification. The MSCA required more detailed information about the toxicity data presented for read across.

The DS clarified reliability of some studies and presented additional toxicity studies for soluble iron salts.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider that ferric pyrophosphate is an inorganic substance that hydrolyse but do not degrade.

Bioaccumulation

RAC is of the opinion that iron ions and orthophosphate ions are essential for aquatic species and potential for bioaccumulation is not expected.

Environmental transformation of metals or inorganic metals compounds

Ferric pyrophosphate is insoluble compound which presents iron complex with pyrophosphate anion. In aqueous solution it slowly hydrolyses to iron(III) and orthophosphate ions. Iron is one of the basic metals occurring in the aquatic environment and it is considered a microelement with regard to live organisms. This metal has a broad range of applications that, together with factors conditioning its chemical transitions, results in the occurrence of many iron species in surface waters. The most common oxidation states of iron in water are the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) ions, although other forms may be present in organic and inorganic complexes. In surface waters, iron is generally present in the ferric state; in reducing waters, the ferrous form can persist. Iron (Fe) is an essential micronutrient for marine organisms, and it is now well established that low Fe availability controls phytoplankton productivity, community structure, and ecosystem functioning in vast regions of oceans. The biogeochemical cycle of Fe involves complex interactions between lithogenic inputs (atmospheric, continental, or hydrothermal), dissolution, precipitation, scavenging, biological uptake, remineralization, and sedimentation processes. Each of these aspects of Fe biogeochemical cycling is likely influenced by organic Fe-binding ligands, which complex more than 99% of dissolved Fe. Orthophosphate is essential micronutrient ensuring functioning and biodiversity of aquatic species. Increased orthophosphate concentrations are responsible for algal blooms and dissolved oxygen depletion.

Aquatic toxicity

Ferric pyrophosphate hydrolyses in aqueous solution releasing Fe^{3+} ions and pyrophosphate ions which further dissociate to orthophosphate ions.

Experimental toxicity studies are available for all three trophic levels for ferric pyrophosphate and results showed that for all levels acute toxicity LC_{50}/EC_{50} values are above its solubility in aqueous solution. For chronic toxicity, experimental results are available for fish and invertebrates for plant protection product containing 3% ferric pyrophosphate (BW01 GB formulation). Obtained results indicate chronic toxicity above solubility values.

RAC supports the DS proposal to classify ferric pyrophosphate using weight of evidence approach and read across data from suitable iron and phosphate compounds. The acute and chronic toxicity of released PO_4^{3-} is based on the toxicity data available for $CaHPO_4$ and acute and chronic toxicity for Fe^{3+} toxicity is based on data available for soluble iron salts ($FeCl_3.6H_2O$ and $FeSO_4.7H_2O$).

RAC agrees with the DS calculation of acute ERVs:

Acute $ERV_{ferric\ pyrophosphate}$ = 3.7 x (745.21/223.36) = 12.34 mg/L (around pH 8)

Acute $ERV_{ferric\ pyrophosphate}$ = 16.6 x (745.21/223.36) = 55.38 mg/L (around pH 7)

Acute $ERV_{ferric\ pyrophosphate}$ = 1.29 x (745.21/223.36) = 4.30 mg/L (around pH 6)

RAC agrees with the DS calculation of chronic ERVs:

Chronic $ERV_{ferric\ pyrophosphate}$ = 0.56 x (745.21/223.36) = 1.87 mg/L

Chronic $ERV_{ferric\ pyrophosphate}$ = 5.2 x (745.21/223.36) = 17.35 mg/L

All calculated ERVs are above 1 mg/L. Following the CLP guidance, RAC agrees with the DS that ferric pyrophosphate **does not warrant classification for acute and chronic aquatic hazards**.

12 EVALUATION OF ADDITIONAL HAZARDS

Hazardous to the ozone layer

Due to its low volatility, it is highly unlikely that ferric pyrophosphate can deplete the stratospheric ozone layer. A substance shall be classified as Hazardous to the Ozone Layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer. The low volatility of ferric pyrophopshate precludes an ozone-layer-depleting potential.

The available evidence concerning properties of ferric pyrophosphate and its predicted environmental fate and behaviour indicate that it may not present a danger to the structure and/or the functioning of the stratospheric ozone layer. The physicochemical properties of ferric pyrophosphate do not suggest that this substance will be hazardous to the ozone layer.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The DS concluded that ferric pyrophosphate highly unlikely depletes the stratospheric ozone layer due to its low volatility.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

RAC agrees with the DS that, on the basis of the properties of ferric pyrophosphate, there is no indication of it posing a hazard to the structure and/or the functioning of the stratospheric ozone layer.

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

J. Walker, 2009, Tetrairon tris(pyrophosphate): determination of melting/freezing temperature and water solubility.

M. Włodarczak, 2012, Determination of physiochemical properties of the test material Ferric pyrophosphate.

M. Włodarczak, 2015, Determination of physiochemical properties of the test material Ferric pyrophosphate.

Anonymous 1, 2013, Acute Oral Toxicity Study on Rats – Fixed Dose Method.

Anonymus 2, 2012a, Tetrairon tris(pyrophosphate): acute oral toxicity in the rat - fixed dose method.

Anonymus 3, 2013, Acute inhalation toxicity study in rats with ferric pyrophosphate.

Anonymus 4, 2012, Tetrairon tris (pyrophosphate): Acute inhalation toxicity (nose only) study in the rat.

Anonymus 5, 2013, Ferric pyrophosphate Acute Toxicity: Dermal Irritation/Corrosion.

Anonymus 6 2012a, Determination of skin irritation potential using the episkintm reconstructed human epidermis model.

Anonymus 6, 2012b, Tetrairon tris(pyrophosphate): In Vitro skin corrosion in the episkin reconstructed human epidermis model.

Anonymus 7, 2013, Ferric pyrophosphate Acute Eye Irritation/Corrosion.

Anonymus 6, 2012c, Tetrairon tris(pyrophosphate): The bovine corneal opacity and permeability assay.

Anonymus 2, 2012b, Tetrairon tris(pyrophosphate): Acute eye irritation in the rabbit.

Anonymus 2, 2011, Local lymph node assay in the mouse.

Nikhil S. Sathe, 2014, Bacterial Reverse Mutation Test of Ferric Pyrophosphate using Salmonella typhimurium Tester Strains.

Anonymus 8, 2014, In vitro Mouse Lymphoma Forward Mutation Assay of Ferric Pyrophosphate by using Mouse Lymphoma (L5178Y TK+-) Cell Line.

Anonymus 9 2013, Assessment of the mutagenic potential of ferric pyrophosphate in micronucleus test in vitro on human lymphocytes (OECD TG 487).

Anonymus 10, 2014, Mammalian erythrocyte micronucleus test with using animals from repeated dose 90-day oral toxicity study in rodents.

Anonymus 11, 2013, Repeated Dose 28-Day Oral Toxicity Study in Rodents of iron pyrophosphate.

Anonymus 12, 2014, Repeated dose 90-day oral toxicity study in rodents.

Anonymus 13, 2013, Acute toxicity to rainbow trout.

A. Ziółkowska, G. Wickiel, 2013, Aquatic invertebrates short-term toxicity.

I. Heisterkamp, 2015, Growth inhibition test on algae.

Anonymous 14,2014, Long-term and chronic toxicity to fish.

J. Winkler, 2014, Daphnia reproduction test.

Additional references

United States Environmental Protection Agency; Reregistration eligibility Document (RED): Iron Salts US EPA 1993

Mineral Information Institute pod red. Draggan S.: Iron. Publication online http://www.eoearth.org/view/article/51cbee407896bb431f696707, 2011

Schulte E.E.: Understanding Plant Nutrients: Soil and Applied Iron, 1992

Bezak-Mazur E., Stoińska R.: The importance of phosphorus in the environment – review article. Archives of Waste Management and Environmental Protection, 2013, 15, pp. 33-42

Fidler M.C., Walczyk T., Davidsson L., Zeder C., Sakaguchi N., Juneja L.R., Hurrell R.F.: A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. British Journal of Nutrition, 2004, 91, pp.107-112

U.S. Food and Drug Administration, 1988, SCOGS-Report Number: 35; 21 CFR Section: 184.1304

Biesinger KE, Christensen GM (1972). Effects of various metals on survival, growth, reproduction and metabolism of Daphnia magna. Journal of Fisheries Research Board of Canada 29: 1691-1700.

Birge WJ, Black JA, Westerman AG, Short TM, Taylor SB, Bruser DM, Wallingford ED (1985). Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warmwater species of aquatic life in the Commonwealth of Kentucky. Memorandum of Agreement No. 5429, Kentucky Natural Resources and Environmental Protection Cabinet.

Nelson (2001) - Iron and colorectal cancer risk: human studies, Nutrition Rev. 2001 May; 59(5):140-8

Sinha S, Rai UN, Chandra P (1994). Accumulation and toxicity of iron and manganese in Spirodela polyrhiza (L.) schieiden. Bulletin of Environmental Contamination and Toxicology. 53(4):610–7.

Torti and Torti (2013) - Iron and cancer: more ore to be mined, Nat Rev Cancer. 2013 May; 13(5): 342–35.

15 ANNEXES

Ferric pyrophosphate_DAR_04_Volume_3CA_B-2

Ferric pyrophosphate_DAR_08_Volume_3CA_B-6

Ferric pyrophosphate_DAR_10_Volume_3CA_B-8

Ferric pyrophosphate_DAR_11_Volume_3CA_B-9

Ferric pyrophosphate_DAR_11_Volume_3CA_B-9

Ferric pyrophosphate_DAR_19_Volume_3CP_BW01_GB_B-9

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TETRAIRON TRIS(PYROPHOSPHATE); FERRIC PYROPHOSPHATE