



Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain

Elemental iron

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Version History

When	What
November 2021	Initial DAR
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B.9. ECOTOXICOLOGY DATA

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1. Acute oral toxicity to Birds

No data for the active substance was submitted.

B.9.1.1.2. Short-term dietary toxicity to birds

No data for the active substance was submitted.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

No toxicity data was submitted.

A number of literature sources are referred to in the risk assessment which are evaluated here. It is noted that the literature sources are not derived from the systematic literature review submitted in support of this application. The period covered by the Elemental Iron literature review is 2006-2017. Therefore it is not certain that the additional data submitted are the only ecotoxicologically relevant data for iron and non-target organisms outside of the period covered by the literature review. In response to a request for further consideration of this point, the applicant has submitted additional information regarding the literature searches performed for data prior to 2006. This is discussed further in the risk assessment section (see Vol 3CP B9).

The applicant submitted examples of where avian commercial feed contain iron ranging from 50-130 mg Fe/kg feed (see section B9.1.1.3/3 of this document). The lowest value was taken (50 mg Fe/kg food) to generate a NOAEL of **5 mg a.s./kg bw/d** - in line with EFSA guidance on converting endpoints from mg a.s./kg food/ppm to mg a.s./kg bw/d¹. This is discussed further in the risk assessment for birds in Vol 3CP B9.

Table 9.1.1.3-1 Iron concentrations in commercial diets according to animal requirements

Bird species	Iron concentration per kg of diet	Reference
Female turkey poults Male turkeys	60 mg	Boling S.D. & Firman J.D., 1997 Boling S.D. & Firman J.D., 1998 Moor <i>et al</i> , 2003
Poultry	62.5 mg	Dieumou F. E 2013
Turkey	130 mg	Eissler & Firman 1996
Chicken & Turkey	50-80 mg	Firman J.D. 1991

¹ EFSA Journal 2009, 7(12) 1438 page 20. A default factor of 0.1 is used.

B.9.1.1.3/1

Study: Nutrient requirements of Chickens and Turkeys. (data from 1984, printed 1991)

Author: Firman, J.

The levels of iron, in mg/kg feed, required for each stage of life are listed in Firman (1991). Overall, the maximum iron requirement for three types of poultry (leghorn chickens, broiler chickens and turkeys) is 80 mg/kg feed. The level of iron required is reduced to about 60 mg/kg feed as the fowl grow to adulthood, and reduces to 50 mg/kg feed during egg laying.

Study: Effects of feather meal on the performance of Turkeys (1996)

Author: Eissler, C. and Firman, J.

In Eissler and Firman (1996) the level of iron used in a feather meal-enriched diet was 130 mg/kg feed, with no apparent effects noted on growth, although the effect of iron was not the objective of the study and reproductive effects were not measured.

Study: Growth Performance, carcass characteristics and economics of production of broilers fed diets with two sources of protein and two levels of wheat offal. (2013)

Author: Dieumou, F., T. Adegbola and U. Doma.

In Dieumou *et al.* (2013) chickens were fed a diet containing 0.25 % vitamin premix containing 25 g of iron, equivalent to 62.5 mg iron/kg feed with no ill effect reported, although the effect of iron was not the objective of the study and reproductive effects were not measured.

Study: Digestible Sulfur Amino Acid Requirement of Male Turkeys During the 12 to 18 week Period. (2003).

Author: Moore, D., K. Baker, K. Thompson, E. Blair and J. Firman

Study: Digestible Lysine requirement of Female Turkeys during the starter period (1998).

Study: A low Protein diet for Turkey poults (1997)

Author: Boling, S. and J. Firman.

In Moore *et al.* (2003) and Boling and Firman (1997; 1998) male and female turkeys were fed a commercial diet containing 60 mg Fe/kg feed with no apparent effects, although the effect of iron was not the objective of the study and reproductive effects were not measured.

HSE Comments

The dataset above, consisting of six articles from the available literature, offers an insight into the amount of iron (in mg Fe/kg feed) commonly consumed by poultry with no apparent ill effects on mortality or growth, though it is noted that the effects of iron on the long term health and reproduction of the birds were not the objective of the studies.

B.9.1.1.3/2

Study Title: Iron metabolism in the Laying Hen (1954)

Author: Ramsay, W. and Campbell, E.

Summary:

Serial determinations of total blood iron and plasma iron were undertaken in a small flock of laying hens throughout a laying season. All eggs were also analysed for iron. In addition, in a separate experiment, laying hens were killed at intervals over the egg laying period and the livers, kidneys and spleens analysed for total and non-haem iron.

Methods:

Brown Leghorn chickens were maintained at the Poultry Research Centre, Edinburgh. The standard stock breeding diet contained FeSO_4 providing 5-6 mg Iron per day. A few birds were fed for 2 months on the same diet without the iron supplement, which lowered the dietary intake of iron by 20 %, but these birds behaved similarly to the others. No attempt was therefore made to present the results of these birds separately.

Birds were observed from the commencement of the laying season at the end of December until the following autumn. All eggs were analysed and at intervals of 3 weeks blood specimens were used for the determination of total iron and plasma iron.

In order to investigate the effects of laying on iron storage organs, a series of laying hens were killed shortly before or after they commenced to lay. Blood specimens and the livers, spleen and kidneys were removed for determination of non-haem and total iron.

Blood specimens were drawn as required (4-5 mL every three weeks). Total blood iron was determined, and haemoglobin concentrations were calculated from these results by assuming that fowl haemoglobin resembles many mammalian haemoglobins in containing about 0.34 % Iron. Plasma iron, Egg iron and Liver, spleen and kidney iron were also determined.

Results:

- Plasma Iron. Until about 10 days before laying, a hens plasma iron concentration lay between 100 and 250 $\mu\text{g}/100\text{ mL}$. Shortly before laying commenced it rose to 500-900 $\mu\text{g}/100\text{ mL}$ and fluctuated narrowly within these high limits as long as eggs were regularly reproduced. Cessation of egg production was accompanied by a return to the original low level.
- Haemoglobin. Blood Haemoglobin concentrations calculated from all of the total iron determinations during the egg laying period ranged from 7.9-9.1 g/100 mL, and before laying ranged from 8.2-12.0 g/100 mL.
- Egg iron. More than 2500 eggs were analysed. The average iron content was 0.88 mg/egg (range: 0.76-1.07 mg/egg)
- Liver, spleen and kidney iron. The livers of non-laying pullets contained an average of 7.7 mg non-haem iron while those of the laying ones contained 5.7 mg. The spleen and kidneys contained much less iron, ranging from 0.14-1.31 mg (mean).

Discussion:

Determinations of egg and plasma iron over a whole season in fifteen birds showed a great elevation of plasma iron just before laying commenced. The high level persisted during the period of regular laying and there was a high correlation between mean plasma iron and mean egg iron.

Total blood iron determinations over the same period suggested that in many hens the onset of laying is preceded by a fall in blood haemoglobin. The lower level persists during the period of regular laying.

Determinations of total and non haem iron in the liver, spleen and kidney over the short period during which hens were coming into lay suggested that there may perhaps be a slight decrease in non-haem iron in the liver at this time, but that the quantity involved is small compared to that utilised for the eggs in the same period.

It was concluded that the demand for iron in the eggs must be met by an increase in the efficiency of iron absorption.

HSE Comments

The above study was submitted in support of the risk assessment. The results show that hens are capable of regulating the level of iron in the blood stream in order to meet the needs of their eggs. It is noted that some hens were not fed an iron-supplemented diet, and that these hens did not display any differences in the results, though it would have been useful to have these hens results highlighted and compared/contrasted with those that received extra iron.

Overall, for the purposes of the risk assessment of iron as an active substance, the study is generally indicative of the differing levels of iron in birds before and during egg laying, and suggests that iron is an essential element in the egg laying process (though birds without iron in their diet were reported not to differ in terms of behaviour from the others). In terms of determining the toxicity of iron, low concentration of iron in the diet fed to the hens (5-6 mg Fe/day) is evidently a standard diet that does not cause adverse effects on breeding, at least for the purposes of commercial rearing of poultry.

B.9.1.1.3/3

Study Title: Serum Iron and its Transport Mechanism in the Fowl (1961)

Author (s): Planas, J., De Castro, S. and Recio J. M.

Summary:

Four species of fowl (*Gallus domesticus*, *Meleagris gallopavo*, *Anas platyrhynca* var. Kaki-Campbell and *Anser anser*) were investigated for serum iron and total iron-binding capacity.

Laying females showed a considerable rise in serum iron, giving values higher than those for males and non-laying females. These values are also different from those which were found in different mammals. This rise was especially observable in ducks and geese, in particular with total iron binding capacity.

Table 1. SERUM IRON, TOTAL IRON-BINDING CAPACITY AND SATURATION COEFFICIENT IN CERTAIN BIRDS

Species	Sex and No. of specimens	Serum iron (γ Fe per cent)	Total iron-binding capacity (γ Fe per cent)	Saturation coefficient (per cent)
<i>Meleagris gallopavo</i>	♂ 10	70 ± 16.2 ($\sigma = 48.7$)	302.1 ± 12.2 ($\sigma = 36.6$)	24.7 ± 5.4 ($\sigma = 16.2$)
	♀ NL 11	104.9 ± 14.5 ($\sigma = 45.2$)	315.2 ± 14.8 ($\sigma = 46$)	33.6 ± 4.4 ($\sigma = 13.9$)
<i>Gallus domesticus</i>	♂ 23	102.5 ± 4.8 ($\sigma = 22.4$)	165 ± 14.2 ($\sigma = 65.5$)	67.8 ± 3.9 ($\sigma = 18.1$)
	♀ NL 23	129 ± 10.4 ($\sigma = 47.8$)	262 ± 15 ($\sigma = 69$)	51.7 ± 4.1 ($\sigma = 18.9$)
	♀ L 16	500 ± 42.4 ($\sigma = 165.5$)	272 ± 21.8 ($\sigma = 85.1$)	198 ± 21.5 ($\sigma = 83.9$)
<i>Anas platyrhynca</i>	♂ 6	159.1 ± 50 ($\sigma = 110.2$)	634.5 ± 54.5 ($\sigma = 120.1$)	25.5 ± 7.3 ($\sigma = 16.1$)
	♀ NL 6	132 ± 18.5 ($\sigma = 40.5$)	472 ± 54.2 ($\sigma = 108.5$)	28.4 ± 2.1 ($\sigma = 4.3$)
	♀ L 19	$1,065 \pm 68$ ($\sigma = 286.6$)	504 ± 32.9 ($\sigma = 131.8$)	227.5 ± 18.1 ($\sigma = 72.7$)
<i>Anser anser</i>	♂ 7	163.8 ± 16.1 ($\sigma = 38.7$)	547.3 ± 25.1 ($\sigma = 60.3$)	29.9 ± 2.3 ($\sigma = 5.5$)
	♀ NL 6	160 ± 10.5 ($\sigma = 23.1$)	561 ± 36.5 ($\sigma = 80.3$)	28.9 ± 1.9 ($\sigma = 4.2$)
	♀ L 1	1.260	705	178.7

NL = not laying.

L = laying.

HSE Comments

The above study is taken from the available literature on iron and submitted in support of the risk assessment. The results show that fowl are capable of regulating the level of iron in the blood stream in order to meet the needs of their eggs.

Overall, for the purposes of the risk assessment of iron as an active substance, the paper is generally indicative of the levels of iron in birds, and that iron is an essential element in the egg laying process. However, without any data on the level of iron fed to the birds used in the study it is of little use in determining the toxicity of iron to birds.

B.9.1.1.3/4

Study: Hemosiderosis and Dietary Iron in Birds (1995)

Author: Dierenfield, E. M. Pinis, and C. Sheppard.

Summary:

Hemosiderosis is characterised by excessive iron storage in body tissues, especially the liver. This results in icterus and ascites with associated liver pathology. Although hemosiderosis has been reported in birds without disease, hepatic iron overload has been associated with disease in a number of avian species kept in zoos including Mynahs, birds of paradise, hornbills and toucans. Whether this is due to genetic factors or dietary factors is subject to debate, as studies elsewhere have shown that either could be the case. In this study, Liver iron concentrations in two types of tropical bird kept in zoos were compared with those of free ranging birds of the same type.

The table below indicates the results from this trial.

TABLE 1

Liver iron concentrations in zoo avifauna known to be susceptible to excessive iron storage¹

Family/Order	n	Liver [Fe] ²
		<i>mg/g wet tissue</i>
Paradisaeidae		
Zoo - dietary Fe unknown	22	5517.6 ± 3797.1
Zoo - dietary Fe 65 ppm	20	4245.8 ± 2958.5
Free-ranging	2	469.0 ± 36.7
Sturnidae		
Zoo - dietary Fe unknown	30	1858.5 ± 1807.5
Zoo - dietary Fe 156 ppm	20	1984.6 ± 1413.9
Free-ranging	24	700.3 ± 125.9
Other Passeriformes		
Zoo	88	500–1000

¹Free-ranging bird comparative data from samples as described in Dierenfeld and Sheppard (1989).

²Values are means ± SD.

The results were taken to reinforce the fact that birds that have low iron diets in the wild were susceptible to iron-storage disease in zoos where a more iron-fortified diet was used.

HSE Comments:

The study indicates that in birds which have evolved to consume a low iron diet, consumption of high concentrations of iron can lead to excessive iron storage that may result in pathogenicity. The use of this study in the context of the risk assessment is discussed further in Vol 3CP B9.

B.9.1.1.3/5

Study: Iron Metabolism in pigeons (1978)

Author: Ramis, J. and Planas, J.

Summary:

A sample of 118 urban pigeons were analysed for several haematological parameters such as haemoglobin concentration, haematocrit, erythrocyte number, reticulocyte concentration, plasma iron and total iron binding capacity, and compared with the same results for turkeys, ducks and chickens.

Organ distribution of ^{59}Fe following intravenous injection at 0.5-1.5 $\mu\text{Ci/kg}$ was analysed during a period from 5 minutes up to 120 days (19 separate occasions) in groups of 4 pigeons. Each group was killed at the appropriate timepoint and the organs analysed for iron content – liver, spleen, breast muscle, leg muscle, heart, ribs and sternal keel, femur and tibia.

Results

TABLE III. Plasma iron turnover (PIT) expressed as 100 ml blood $^{-1}$ and as Kg of body weight $^{-1}$. (L = laying).

Species Sex No specimens	Body Weight Kg	PIT mg Fe. 100 ml blood . day	PIT mg Fe. Kg b.w. . day
Pigeon			
♂ 23	0.30 \pm 0.02	3.8 \pm 1.4	6.8 \pm 2
♀ 13	0.27 \pm 0.02	4.3 \pm 0.9	8.3 \pm 2
*Duck			
♂ 8	2.8 \pm 0.5	3.2 \pm 0.8	3.03 \pm 1.7
♀ 4	1.0 \pm 0.08	6.05 \pm 2.3	9.7 \pm 4.5
♀ L 8	1.5 \pm 0.01	5.6 \pm 2.9	4.4 \pm 1.9
*Turkey			
♂ 7	3.1 \pm 0.4	3.9 \pm 1	2.2 \pm 0.7
♂ 5	10.6 \pm 1.7	3.4 \pm 1	0.9 \pm 0.2
♀ 5	4.4 \pm 0.6	2.4 \pm 0.3	1.02 \pm 0.1
♀ L 4	7.3 \pm 0.7	4.9 \pm 0.9	2.5 \pm 1
†Domestic Fowl			
♂ 7	2.0 \pm 0.5	2.8 \pm 0.7	1.6 \pm 0.5
♀ 7	1.5 \pm 0.3	2.9 \pm 0.9	1.9 \pm 0.7
♀ L 16	3.0 \pm 0.7	5.5 \pm 0.2	2.6 \pm 1

Data calculated from: *Balasch and Planas [1972], and †Planas and Balasch [1970].

TABLE IV. Percentage of total injected ^{59}Fe distributed among the whole organs at different time intervals. Tibia, femur and leg muscle values correspond to both legs. Each value is the mean of 4 specimens and in some cases the standard deviation is shown.

	5 min	10 min	20 min	30 min	1 h	3 h	5 h	12 h	1 day
Blood	127 ± 5.5	109 ± 22	92 ± 47	73.76 ± 10	53.8 ± 12	69.23 ± 14	51.7 ± 8.42	73.4 ± 12	77.78 ± 25
Spleen	0	0.0025	0.01	0.08	0.08	0.09	0.27	0.09	0.13
Liver	0	0.09	0.17	0.60	0.69	0.71	5.66	—	3.33
Tibio tarsus	1.553 ± 0.44	1.797 ± 1.09	2.274 ± 0.39	2.531 ± 0.42	2.786 ± 1.08	3.818 ± 1.05	4.829 ± 1.04	2.76 ± 1.47	3.04 ± 0.86
Femur	2.291 ± 0.88	2.358 ± 0.09	2.992 ± 1.10	4.536 ± 1.19	5.703 ± 2.87	5.794 ± 1.71	7.792 ± 1.30	3.924 ± 1.62	3.196 ± 1.47
Rib	0.315	0.258	0.296	0.248	0.148	0.250	0.149	0.301	0.139
Sternal-Keel	0.891	0.808	0.817	0.931	—	0.706	0.345	0.805	0.298
Breast muscle	1.06	2.11	1.61	1.82	—	2.54	1.18	0.53	1.01
Leg muscle	0	0.44	0.13	0	0	0.04	0.18	0	0.06
Heart	0.13	0.11	0.18	0.20	0.20	0.12	0.19	0.10	0.15
Gonad	0.030	0.104	0.116	0.113	0.102	0.019	0.070	0.021	0.021

	2 days	3 days	4 days	5 days	10 days	20 days	40 days	60 days	80 days	120 days
Blood	98.7 ± 2	97.7 ± 17	94.7 ± 30	83.8 ± 7.8	83 ± 15	79.2 ± 6.08	51.08 ± 11.08	48.01 ± 12.66	—	—
Spleen	0.24	0.30	0.44	0.25	0.64	0.22	0.41	1.30	0.23	0.17
Liver	2.25	2.28	2.33	2.24	2.01	0	5.75	4.16	2.01	1.18
Tibio tarsus	0.863 ± 0.52	0.797 ± 0.11	0.679 ± 0.58	0.456 ± 0.21	0.705 ± 0.25	0.33 ± 0.08	0.494 ± 0.04	0.33 ± 0.03	0.20 ± 0.09	0.18 ± 0.06
Femur	1.273 ± 0.93	1.059 ± 0.31	1.022 ± 0.86	0.666 ± 0.15	0.667 ± 0.25	0.38 ± 0.05	0.606 ± 0.05	0.41 ± 0.03	0.31 ± 0.15	0.25 ± 0.08
Rib	0.107	0.097	0.151	0.114	0.115	0.06	0.206	0.05	0.11	0.03
Sternal-Keel	0.556	0.380	0.430	0.234	0.445	0.11	0.299	0.11	0.09	0.07
Breast muscle	1.76	1.99	1.39	0.14	0.68	1.99	1.42	0.72	1.18	1.73
Leg muscle	0	0.12	0.57	0	0	0	0.03	0	0.19	0.29
Heart	0.13	0.15	0.06	0.10	0.06	0.08	0.14	0.20	0.10	0.23
Gonad	0.041	0.055	0.049	0.022	0.037	0.01	0.148	0.03	0.11	0.02

Discussion

An inversely proportional relationship was observed between plasma iron turnover (PIT) and body weight amongst pigeons, chickens, turkey and geese. The mean clearance rate observed in pigeons was 20-21 minutes and similar to that of young pullets (18-25 mins), whilst that of ducks was 33-51 mins and turkeys were 33-40 mins. The amount of iron in the pigeon's bloodstream increased shortly after injection with the radioactive Fe, peaking on the 3rd day, before steadily declining until the 40th day. The amount of iron in the spleen and liver displayed the inverse to the blood, with a rapid increase around the 40th day, when the blood exposed to the radioactive iron injection was absorbed by these organs.

HSE comments

The study is generally descriptive of the iron metabolism in pigeons, which was shown to be faster than in larger birds. However as no investigation was made regarding potential toxic effects of the injected iron, the study does not provide data relevant to the resolution of the risk assessment.

B.9.1.1.3/6

Study: Iron Metabolism in Mynah birds (*Gracula religiosa*) resembles human hereditary haemochromatosis (2003)

Author: Mete, A., Hendricks, H., Klaren, P., Dorrestein, G., van Dijk, J., and Marx, J.

Summary:

Mynah birds, a species susceptible to iron overload (caused by high uptake and retention of dietary iron), were compared to non-susceptible avian species (chickens) by evaluating iron uptake at the intestinal absorptive cell level. Enterocytes from mynahs and chickens were isolated and uptake of Fe (II) and Fe (III) was studied in vitro. It was found that Fe (III) uptake was much lower than Fe (II) uptake in both species, but Fe (II) uptake was higher in Mynahs than in chickens. Liver iron present in hepatocytes was 10-fold higher in Mynahs than in chickens. The susceptibility of Mynah birds was concluded to be due to intestinal iron uptake despite hepatic iron accumulation, implicating a 'mis-sensing' of body iron similarly to human hereditary haemochromatosis.

In Human Hereditary Haemochromatosis, excess iron accumulates in the liver and other parenchymal organs, causing tissue damage and resulting in fibrosis, cirrhosis and hepatocellular carcinoma. The cause of excess iron is the relatively high absorption of iron from the diet with respect to needs. Mutations in genes encoding for iron proteins responsible in the ‘sensing’ mechanism have been identified to be the causes of the condition.

In general, fruit-eating and insect eating birds have a predisposition to develop similar disease – toucans, birds of paradise, etc. Excess iron overload is the most common metabolic disorder of mynahs. It is postulated that the features of iron metabolism in these birds is due to the low iron in their natural habitat, where the duodenal epithelia are ‘programmed’ to take up all the iron available.

HSE Comments

The study indicates that in birds which have evolved to consume a low iron diet, consumption of high concentrations of iron can lead to excessive iron storage that may result in pathogenicity. The use of this study in the context of the risk assessment is discussed further in Vol 3CP B9.

B.9.1.2. Effects on terrestrial vertebrates other than birds

B.9.1.2.1. Acute oral toxicity to mammals

No data was submitted in support of the ecotoxicology dossier; data is drawn instead from Section 6 of the dossier (Toxicology) – please see Vol 3CP Section 9 for endpoints and their use in the ecotoxicological risk assessment.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

No toxicity data was submitted in support of the ecotox dossier; data is drawn instead from Section 6 of the dossier (Toxicology) – please see Vol 3CP Section 9 for endpoints and their use in the ecotoxicological risk assessment.

The applicant submitted some additional data from the available literature in support of the ecotoxicology dossier. It is noted that the literature sources are not derived from the literature review submitted in support of this application; the period covered by the Elemental Iron literature review is 2006-2017. Therefore it is not certain that the additional data submitted are the only ecotoxicologically relevant data for iron and non-target organisms outside of the period covered by the literature review. In response to a request for further consideration of this point, the applicant has submitted additional information regarding the literature searches performed for data prior to 2006. This is discussed further in the risk assessment section (see Vol 3CP B9).

B.9.1.2.2/1

Title: Laboratory Rodent Diet 5001* (no date)

Author: LabDiet (www.labdiet.com)

Summary:

A description of a commercial laboratory rodent diet (LD5001) with feeding directions. The diet is designed for life-cycle nutrition. The amount of iron listed in the diet is 240 mg/kg. It is anticipated that a rat will eat 15-30 g of food daily depending on size, and a mouse will eat 5-8 g of food daily depending on size. The product claims to be the standard of biomedical research for over 70 years.

HSE Comments

The submitted article is a list of ingredients and chemical composition of commercial rodent diet, which contains 240 mg iron/kg food. The implications of this for the risk assessment are discussed in Vol 3CP Section 9.

B.9.1.2.2/2

Title: AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet (1993)

Author: Reeves, P., Nielson, F., Fahey, G.

Summary:

Changes to a standard commercial rodent diet are discussed in light of advances in scientific knowledge. The amount of iron in two rodent diets (AIN-93G and AIN-93M) was 45 mg/kg diet. It is suggested that cheaper sources of fibre contain higher concentrations of iron.

HSE Comments

The submitted article is a list of ingredients and chemical composition of commercial rodent diet, which contains 45 mg iron/kg food. The implications of this for the risk assessment are discussed in Vol 3CP Section 9.

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

No information submitted.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No information submitted.

B.9.1.5. Potential for endocrine disruption

No information submitted.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

No information submitted.

B.9.2.2. Long-term and chronic toxicity to fish

No information submitted.

B.9.2.3. Potential for endocrine disruption

No information submitted.

B.9.2.4. Acute toxicity to aquatic invertebrates

No information submitted.

B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates

No information submitted.

B.9.2.6. Effects on algal growth

No information submitted.

B.9.2.7. Effects on aquatic macrophytes

No information submitted.

B.9.2.8. Further testing on aquatic organisms

No information submitted.

B.9.3. EFFECTS ON ARTHROPODS**B.9.3.1. Effects on bees**

No acute or long term toxicity studies with the active substance were submitted.

The following literature article was submitted in support of the risk assessment.

B.9.3.1/1

Study Title: A comparison between the mineral content of flower and honeybee collected pollen of selected plant origin (*Helianthus annuus* L. and *Salix* sp.). 2011.

Author(s): Stanciu, O., L. Marghitas, D. Dezmirean and M. Campos

Summary:

The macro and micro nutrient content of *Helianthus annuus* L. and *Salix* sp. flower pollen in comparison with bee pollen (a mixture of flower pollen, nectar and bee salivary substances) of the same plant origin was determined.

Samples of bee pollen were purchased commercially and originated from Romania, having been collected in 2009. The flower pollen was collected by hand (also from Romania). The bee pollen loads were first separated by colour from the complex mixtures of pollen pellets coming from different species of plants, resulting in monochromatic pollen loads with uniform colour. Microscopic examination was used to identify the origin of the pollen within the bee pollen pellets. In each microscopic preparation, pollen was determined, when possible, into genus, species or family.

The pollen types were analysed for mineral content using an Atomic Absorption Spectrophotometer. A 2 g sample was analysed in triplicate.

Table 1. The macro and micromineral content of flower and honeybee collected pollen of selected plant origin*

Botanical name of floral species	Mineral content (mg kg ⁻¹)				
	K	Ca	Mg	Fe	Zn
Honeybee collected pollen					
<i>Helianthus annuus</i> L.	3246.50 ±38.96	1409.79 ±28.20	376.94 ±4.83	27.42 ±1.37	31.61 ±1.58
<i>Salix</i> sp.	5421.85 ±65.06	2630.67 ±52.61	1008.28 ±20.17	122.87 ±6.14	40.06 ±2.00
Flower pollen					
<i>Helianthus annuus</i> L.	5902.76 ±70.83	8090.28 ±91.81	1336.60 ±26.73	2872.89 ±93.64	70.28 ±3.51
<i>Salix</i> sp.	8686.64 ±94.24	2895.28 ±57.91	2193.13 ±43.86	325.29 ±16.26	79.74 ±3.99

*Samples analyzed in triplicate.

The significantly richest ($p < 0.05$) samples, with respect to the iron content, belonged to the flower pollen of *Helianthus annuus* L., with values of 2872.89 mg kg⁻¹ (Table 1). In comparison, the unifloral bee pollen originated from the same plant species contained only 27.42 mg kg⁻¹ iron. The iron content of *Salix* sp. pollen samples registered values of 325.29 mg kg⁻¹ (flower pollen) and 122.87 mg kg⁻¹ (bee pollen). Iron values in multi-floral bee pollen have been previously reported in the range of 40.4-136.1 mg/kg in Poland samples, 74.3-365.9 mg/kg in South Korea samples and 59.0- 182.3 mg/kg in China samples. Commercial bee pollen from Spain was reported to contain 40 mg iron/kg. However, bee pollen collected in Columbia (GG Salamanca *et al.*) contained as much as 2576 mg iron/kg.

The decrease in mineral concentration in bee pollen samples in comparison with flower pollen was postulated to reflect dilution due to the addition of regurgitated honey, which contains nectar and honeybee salivary substances, added by the bees, in order to prepare the pollen pellets and to transport them in the hive.

HSE Comments

The above study indicates that flower pollen from *Salix* sp. and *Helianthus annuus* L. can contain as much as 325.29 and 2872.89 mg iron/kg pollen, respectively, and that in comparison bee pollen from the same species contains a much lower concentration (122.87 and 27.42 mg iron/kg bee pollen, respectively). This was proposed by the study authors to be because the flower pollen is diluted by the bees with saliva to make bee pollen pellets. The sample size appears to have been small (3x 2 g pollen per species) but correlates roughly with samples from other countries as discussed in the study. Evidently bee pollen can contain up to 2576 mg iron/kg, but this varies widely depending on the origin of the bee pollen. European (Poland, Romania, Spain) bee pollen samples quoted range from 27.42 mg iron/kg pollen to 136.1 mg/kg pollen.

B.9.3.2. Effects on non-target arthropods other than bees

No information submitted.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

No information submitted.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

No information submitted.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

No information submitted.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS**B.9.6.1. Summary of screening data**

No active substance data was submitted.

B.9.6.2. Testing on non-target plants

No active substance data was submitted.

The following literature articles were submitted in support of the risk assessment.

B.9.6.2/1

Title: Iron Homeostasis in Plants – a Brief Overview. 2017.

Author(s): Connorton, J., J. Balk, and J. Rodriguez-Celma

Summary

Iron is considered an important nutrient in plants, involved in electron transfer functions, chemical transitions such as hydroxylations, radical mediated rearrangement and de/hydration reactions. Iron limitation severely affects plant growth and iron is often included in agricultural fertilisers, but excess iron is toxic to cells and so organisms have evolved ways of metabolising and storing iron. The article discusses the various methods by which plants take up, distribute and store iron (in the form of Fe^{2+} and Fe^{3+}). It also discusses regulation of iron homeostasis, which is important to ensure enough iron reaches the tissues where it is needed without accumulating to toxic levels. Several post-transcriptional mechanisms have been observed that rapidly stop iron uptake. This chiefly revolves around the ubiquitination of iron-transport proteins in the endodermis of roots in response to a detected excess of iron in the cell.

HSE Comments

The paper is broadly indicative of the importance of iron in plant homeostasis and illustrates how plants are capable of responding to excess iron levels. It provides supplemental information for the purposes of risk assessment of the active substance.

B. 9.6.2/2

Title: Phytotoxicity of ionic, micro- and nano-sized iron in three plant species (2015).

Authors(s): Libralato, G., Costa Devoti, A., Zanella, M., Sabbioni, E., Micetic, I., Manodori, L., Pigozzo, A., Manenti, S., Groppi, F. and Volpi Ghirardini, A.

Summary

Effects of differing concentrations of ionic (FeCl_3), micro- and nano-sized zerovalent iron (nZVI) on three macrophyte species were observed. The species of plants were *L. sativum*, *S. alba* and *S. saccharatum*. The toxicity indicators were seed germination, seedling elongation, germination index and biomass. The concentrations tested were 1.29-1570 mg/L FeCl_3 , 1.71-10.78 mg/L micro-sized iron and 4.81-33,560 mg/L nano-iron.

Exposure effects were also observed by optical and transmission electron microscopy.

Results showed no significant phytotoxic effects for both micro- and nano-sized iron. Ionic iron showed slight toxicity at 1570 mg/L and no median effect concentrations could be determined. The most concentrated treatments showed black spots and coating on roots of all three species.

HSE Comments

The study treated the plants with a spray solution of different forms of iron, and does not really represent the conditions of exposure in the case of elemental iron (formulated as a pellet). However, these solutions only caused minor phytotoxic effects at the highest treatment rate. It is noted that the study considers that the safety of nano-sized zerovalent iron (nZVI) should be confirmed with further study, particularly medium and long-term exposure assays. Overall the study is not considered to be useful for the purposes of risk assessment.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No information submitted.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Iron is a naturally occurring element and the amount of iron added to the soil is several orders of magnitude smaller than the natural content commonly found in soils. UK Environmental Fate and Behaviour specialists estimate background levels 2000-50 000 mg Fe/kg soil, and the worst-case PEC_{soil}(accumulation) from the requested GAP for elemental iron is estimated to be 12.8 mg a.s./kg soil. Therefore the amount of iron that sewage treatment plants are naturally exposed to will not be significantly increased by application of elemental iron according to the requested GAP. On this basis no data is required or requested and an acceptable risk to sewage treatment processes is concluded.

B.9.9. MONITORING DATA

No information submitted.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

No information submitted.

B.9.11. REFERENCES RELIED ON

B.9.11.1. Literature Review

The applicant has conducted a literature review according to the requirements of Regulation (EU) No. 283/2013, which refers to Article 8(5) of Regulation (EC) No 1107/2009. The applicant also states that the search was conducted following the ECPA 'Technical guidance on the application of the EFSA Guidance Document "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" '.

The applicant conducted a single literature review encompassing all areas of the assessment: Toxicology; Ecotoxicology; Metabolism; Residues and Environmental Fate and Behaviour. The search was designed to capture any data published between January 2006 and November 2016. A top-up search was conducted to cover the period from November 2016 to December 2017.

The table below shows the databases used by the applicant to perform the search. The HSE considers that a reasonable number of databases have been used and several of these are directly relevant to Ecotoxicology. In particular, the 'AGRICOLA', 'Analytical Abstracts', 'CAB ABSTRACTS', and 'Environment Abstracts' cover environmental related topics.

Table 3CA B.9.11.1-1 Databases used/ search engines

Database	Applicant's Justification	Subject coverage
AGRICOLA	Consists of worldwide literature citations for journal articles, monographs, proceedings, theses, patents, translations, audio-visual materials, computer software, and technical reports pertaining to all aspects of agriculture and related fields.	<ul style="list-style-type: none"> -Agriculture (general) - Agriculture (products, engineering, information systems) - Animal sciences -Biotechnology -Botany - Chemical conservation - Cytology - Agricultural economics, energy, entomology, and history - Farm management - Feed science -Fertilisers - Fibre and textiles - Food and nutrition - Forestry - Horticulture - Human ecology - Human nutrition - Hydrology - Microbiology - Natural History - Natural resources - Pesticides - Physiology - Plant sciences - Pollution -Public health - Rural sociology - Soil sciences - Veterinary medicine - Water quality - Weather and climate - Wildlife - Zoology
Analytical Abstracts	Covers all aspects of analytical chemistry in a wide variety of areas including general applications, biochemistry and clinical chemistry, industrial and applied science, environmental science, agriculture and food, pharmaceuticals and instrumentation.	<ul style="list-style-type: none"> - General - Inorganic - Organic - Industrial - Biochemical - Pharmaceutical - Food - Agricultural and environmental - Computer handling of analytical data - Instrumentation
BIOSIS ® Toxicology	Subset of BIOSIS ® Previews with a focus on toxicology and related topics. Records are drawn from journal articles, conference papers, monographs and book chapters, notes	<ul style="list-style-type: none"> -Agriculture - Bacteriology -Biochemistry - Biophysics

Database	Applicant's Justification	Subject coverage
	letters, and reports, as well as original research. U.S. patent records are also included.	<ul style="list-style-type: none"> - Biotechnology - Botany - Cell biology - Clinical medicine - Drugs - Environmental biology - Environmental science - Experimental medicine - Genetics - Immunology - Microbiology - Nutrition - Occupation health - Parasitology - Pathology - Pharmacology - Physiology - Public health - Radiation Biology - Systematic biology - Veterinary science - Virology
CAB Abstracts	Coverage of worldwide literature on agriculture and allied fields, including veterinary medicine, human nutrition, horticulture, forestry, leisure, recreation, recreation and tourism, crop science, crop protection, breeding and genetics, animal production, animal nutrition, parasitology, soils, land use, agricultural engineering, agricultural economics, and biotechnology. Publication types are journals, monographic series, theses, technical reports, conferences, selected patents, books and annual reports.	<ul style="list-style-type: none"> - Agricultural biotechnology - Agricultural economics and rural sociology - Agricultural engineering - Animal health and veterinary medicine - Animal production and genetics - Biodeterioration and biodegradation - Crop production - Crop protection - Dairy science - Environmental degradation, conservative and amelioration - Forestry - Genetic resources - Horticulture - Human nutrition and diet-related disorders - Human parasitic diseases - Leisure, recreation and tourism - Plant breeding and genetics - Postharvest science - Rural development - Soil science - Sugar industry
Embase ®	Bibliographic coverage of literature on drugs and pharmacology and all other aspects of human medicine and related discipline. Embase is a key resource for biomedical evidence, from published, peer-reviewed literature, in-press publications and conference abstracts.	<ul style="list-style-type: none"> - Drug research - Pharmacology - Pharmacoeconomics - Pharmaceuticals - Toxicology - Human medicine - Basic biological research - Health policy and management - Public, occupational and environmental health - Substance dependence and abuse - Psychiatry

Database	Applicant's Justification	Subject coverage
		<ul style="list-style-type: none"> - Forensic science - Biomedical engineering and instrumentation - Medical devices
Environment Abstracts	Encompasses all aspects of the impact of people and technology on the environment and the effectiveness of remedial policies and technologies. The database covers journals, conference papers and proceedings, special reports from international agencies, non-governmental organisations, universities, associations and private corporations. Other materials selectively indexed include significant monographs, government studies and newsletters.	<ul style="list-style-type: none"> -Agriculture - Air pollution -Control technologies -Endangered species - Energy - Environmental design - Environmental education - Environmental law and policy - Environmental safety - Geophysical and climate science - Global warming - International environmental policy - Land use and pollution - Marine pollution - Noise pollution - Population - Population studies - Radiological contamination - Resource management - Solid and toxic waste - Sustainable development - Toxicological effects - Transportation - waste management - Water pollution - Wildlife/ biodiversity
Medline ®	US National Library of Medicine premier bibliographic database. It contains references to journal articles in life sciences with a concentration on biomedicine and health. This is broadly defined to encompass those areas of the life sciences, behavioural sciences, chemical sciences, and bioengineering need by health professionals and other engaged in basic research and clinical are, public health, health policy development, or related educational activities. Medline also covers life science vital to biomedical practitioners, researchers, and educators, including aspects of biology, environmental science, marine biology, plant and animal science as well as biophysics and chemistry.	<ul style="list-style-type: none"> - Clinic and preclinical medicine - Dentistry - Nursing - Population and reproductive biology - Pharmacology and pharmaceutics - Psychiatry and psychology - Environmental, public and occupational health - Veterinary medicine - Nutrition - Pathology - Anatomy and physiology - Toxicology - Genetics - Microbiology - Pathology - Biomedical technology - Health planning and administration - Space life science
Toxfile ®	Covers the toxicological, pharmacological, biochemical and physiological effects of drugs, pesticides and other chemicals. Typical areas of coverage include drug reactions, chemically induced diseases, carcinogenesis, mutagenesis, teratogenesis, environmental pollution, waste disposal, radiation, and food contamination.	<ul style="list-style-type: none"> - Adverse drug reaction - Air pollution - Animal venom - Antidotes - Carcinogenesis via chemicals - Chemically induced diseases - Drug evaluation - Environmental pollution

Database	Applicant's Justification	Subject coverage
		<ul style="list-style-type: none"> - Food contamination - Metagenesis - Occupation - Pesticides - Radiation - Teratogenesis - Toxicology - Waste disposal
Toxicology Abstracts	Covers issues from social poisons and substance abuse to natural toxins, from legislation and recommended standards to environmental issues.	<ul style="list-style-type: none"> - Pharmaceuticals - Food, additives and contaminants - Agrochemicals - Cosmetics, toiletries and household products - Industrial chemicals - Metals - Toxins and other natural substances - Social poisons and drug abuse - Polycyclic hydrocarbons - Nitrosamines and related compounds - Radiation and radioactive materials - Methodology - Legislation and recommended standards
TOXLINE ®	Toxicology reference database that provides bibliographic information for journal articles on the effects of drugs and other chemicals.	<ul style="list-style-type: none"> - Biochemistry - Pharmacology - Physiology - Toxicology
TRACE	Includes information from peer-reviewed toxicology and nutrition journals as well as secondary sources and websites.	<ul style="list-style-type: none"> - Chemical toxicology

Search strategy and terms

The applicant implemented a single concept search strategy, using the CAS RN number (7439-89-6) for iron, its synonyms and name fragments, along with the form-specific term “powder”. This approach captured all data for all subject areas in a single search rather than using separate focused searches. Either approach is acceptable according to the EFSA 2011 guidance for submission of scientific peer-reviewed open literature.

Table 3CA B.9.11.1-2 Search terms used for the single concept search strategy for elemental iron

Subject areas	Search terms
Toxicology, metabolism, residues, environmental fate and behaviour, ecotoxicology.	(“7439-89-6” or “iron” or “Ancor B” or “Ancor en 80/150” or “Armco iron” or “Atomel 28” or “Atomel 300M200” or “Atomel 500M” or “Atomel 95” or “Atomiron 44MR” or “Atomiron 5M” or “Atomiron AFP 25” or “Atomiron AFP 5” or “ATW 230” or “ATW 432” or “ XXXXXXXXXX iron” or “DSP 1000” or “DSP 1288” or “DSP 135” or “DSP 135C” or “DSP 138” or “EF 1000” or “EF 250” or “EFV 200/300” or “EFV 250” or “EFV 250/400” or “EO 5A” or “Ferronyl” or “Ferrous iron” or “Ferrovac E” or “Ferrum” or “GS 6” or “ XXXXXXXXXX EH” or “NC 100” or “PZh-1M3” or “PZh-2” or “PZh1M1” or “PZh2M” or “PZh2M1” or “PZh2M2” or “PZh3” or “PZh3M” or “PZh4M” or “PZhO” or “Remko” or “SUY-B 2” or “E1UOL152H7”) AND (powder)

The HSE considered that the specific term for the active substance i.e. “elemental iron” should be included in the search strategy for completeness. Furthermore, the HSE considered that as pure iron (Fe) is highly likely to form ionic compounds in the presence of water and oxygen under environmental conditions, this should be taken into consideration in the search terms selected. The following question was asked:

Please include the search terms “ferric” and “elemental iron” in the search strategy.

Specific points to address in reply to HSE CRD request: It is not considered appropriate to exclude all forms of iron other than pure iron powder from the search. As you have noted in the dossier, elemental iron (Fe) is highly likely to oxidise to ferric iron (Fe 3+) in the natural environment and oxides or hydroxides under mammalian physiological conditions. Therefore, the HSE evaluator considers the term “ferric” should also be included in the search strategy. Please also include the specific name of the active substance for which approval is being sought (elemental iron).

Applicant’s response:

“Preliminary searches on the term “elemental iron” for the date range specified in the existing Literature Review Report (LRR) (excluding references already identified by the searches described in the LRR) indicate that slightly more than 500 references would require a rapid filter for potential relevance.

However, similar searches using the term “ferric” returns nearly 80,000 references requiring rapid filter. This would be a substantial undertaking requiring several months to consider the titles only and produce a “short-list” of potentially relevant references. (Please note that this is a preliminary stage, and further work would subsequently be required to evaluate the “short-list”, on the basis of abstract and/or full text, for relevance and reliability)

It may be worth considering at this stage that “ferric” is not a particularly useful search term, in that it will not necessarily identify references relevant to iron in its 3+ oxidation state. For example, ferric oxide would (arguably) be more commonly referred to as “iron oxide”; ferric hydroxide as “iron hydroxide”; therefore, searches for “ferric” would not necessarily identify all the publications that use those terms to identify the test substance. To avoid this pitfall, we would recommend a different approach – this is, the identification of specific compounds and/or physical forms of interest, for which the searches to be undertaken would include CAS Registry Numbers.

We would be pleased to discuss this with you and hear your opinion about the above.”

The HSE has requested that the additional 500 references identified in a preliminary search for the specific term ‘elemental iron’ are considered in the literature review for completeness. The results of the search process for the specific term ‘elemental iron’ are included in table 3CA B.9.11.1-6. The HSE accepts that including the references for ‘ferric’ would be cumbersome and not materially add to the assessment. The more mobile and consequently more bioavailable and toxic form of iron, ferrous iron, is included in the search.

Initial rapid assessment

The applicant carried out an initial rapid assessment of relevance, using the titles of publications alone. Anything considered to have potential relevance (including tenuous, fleeting or even undecipherable relevance) to the fields of environmental fate, toxicology, ecotoxicology, metabolism or residues, were not rejected at this stage.

Criteria for relevance

Of those publications deemed to be of potential relevance, the applicant considered the abstract to conclude whether the publication was either ‘relevant’ or ‘reliable’. For some studies, the applicant considered it necessary to view the full text in order to come to a conclusion. The applicant used the following criteria for relevance when selecting studies related to ecotoxicology

- Appropriate, well defined test material (excluding salts and other iron compounds)
- Appropriate test species (i.e. a species able to inform with respect to ecotoxicology endpoints, including non-standard species if these can be used to provide supplementary information).
- Test substance is applied alone or as part of a solo formulation (mixtures and combined toxicity studies are excluded)

- Test conditions representative of European geoclimatic conditions
- Information that informs or partially informs data requirements, relating to ecotoxicology.

The HSE considers the relevance criteria acceptable.

Criteria for reliability

The following reliability criteria were used to assess any ecotoxicology articles that were deemed relevant. The applicant used the reliability indicators based on the guidance of Mensink et al. (2008).

Table 3CA B.9.11.1-3 Reliability scores used to assess relevant environmental fate and behaviour studies

Reliability indicator	Description	Definition
Ri 1	Reliable without restrictions	All critical reliability criteria for this study are fulfilled. The study is well designed and performed, and it does not contain flaws that affect the reliability of the study.
Ri 2	Reliable with restrictions	The study is generally well designed and performed, but some minor flaws in the documentation or set-up may be present.
Ri 3	Not reliable	Not all critical reliability criteria for this study are fulfilled. The study has clear flaws in the study design and/ or how it was performed.
Ri 4	Not assignable	Information needed to assess the study is missing. This concerns studies that do not give sufficient experimental details and that are only listed in abstracts or secondary literature (books, reviews etc.) or studies of which the documentation is not sufficient for assessment of reliability for one or more vital parameters.

The HSE is not familiar with the reliability scores proposed by Mensink et al. (2008) and therefore asked the following question to the applicant:

Please explain and justify the use of the reliability scores used for Environmental Fate and Ecotoxicology i.e. Mensink et al. (2008) and Moermond et al. (2016)?

Applicant's response:

"For mammalian toxicology, it is standard industry practice to apply the criteria published by Klimisch et al. (1997) in order to provide a "weighting" as to the reliability of each set of experimental results. However, "mammalian toxicology" is a relatively narrow field (for example, there are significantly fewer commonly-used test species) and these criteria are more difficult to apply meaningfully to studies considering environmental and ecotoxicological aspects of chemical behaviour. This is due to, among other factors, the nature and variety of the methodology that can be employed and the conclusions that can (and cannot) be drawn or inferred. Therefore, for studies in these fields, an adaptation of the Klimisch criteria is typically employed (Mensink et al. 2008; Moermond et al. 2016). This serves the same purpose as the Klimisch criteria, as applied to mammalian toxicology, in that it gives individual studies a "weighting" as to the reliability and significance of the effects observed in the context of environmental fate/ecotoxicity risk assessment."

The HSE accepts this justification. It is noted that in the EFSA Q+A for June 2019, EFSA indicated that the Klimisch criteria can be considered for the assessment of reliability. Therefore, the HSE considers the use of Mensink et al. (2008) which is based on Klimisch to be acceptable.

The number of records retrieved for each stage of the process are shown below for the main review (2006 to 2016) and the top-up review (2016 to 2017) in tables 3CA B.9.11.1-4 and -5 respectively. A total of 14 studies were considered relevant and reliable. Only one of these studies was considered relevant for ecotoxicology. This study has been included in the risk assessment.

The HSE considers the literature review of elemental iron to be acceptable.

Table 3CA B.9.11.1-4 Results of the study selection process for elemental iron (includes Environmental Fate, Ecotoxicology, Residues, Metabolism and Toxicology) covering the period from 2006 until November 2016

Stage of study selection process (main review)	Number of summary records retrieved
Total number of summary records retrieved:	11,002
Number of summary records excluded from the search results after rapid assessment for relevance:	10,609
Total number of summary records remaining after filtering out those of clear irrelevance and manual de-duplication:	393
Total number of full-text documents assessed in detail:	38
Number of studies excluded from further consideration after detailed assessment for relevance and/or reliability:	24
Number of studies not excluded for relevance or reliability after detailed assessment:	14
Number of studies not excluded for relevance or reliability after detailed assessment, that were relevant to the ecotoxicological assessment:	1

Table 3CA B.9.11.1-5 Results of the study selection process for elemental iron (includes Environmental Fate, Ecotoxicology, Residues, Metabolism and Toxicology) covering the period from 28th November 2016 until 18th December 2017

Stage of study selection process (top-up review)	Number of summary records retrieved
Total number of summary records retrieved:	2145
Number of summary records excluded from the search results after rapid assessment for relevance:	2061
Total number of summary records remaining after filtering out those of clear irrelevance and manual de-duplication:	84
Total number of full-text documents assessed in detail:	6
Number of studies excluded from further consideration after detailed assessment for relevance and/or reliability:	6
Number of studies not excluded for relevance or reliability after detailed assessment:	0
Number of studies not excluded for relevance or reliability after detailed assessment, that were relevant to the ecotoxicology assessment:	0

Upon request of the HSE, the applicant performed an additional literature review for the specific term ‘elemental iron’. The search process used (i.e. databases, relevance criteria and reliability criteria) was identical to that described above for the main literature review. Out of the five records not excluded for relevance or reliability,

none were considered relevant for ecotoxicology. The HSE considers this additional search acceptable and notes this has no impact on the outcome of the ecotoxicological risk assessment.

Table 3CA B.9.11.1-6 Results of the study selection process for elemental iron (includes Environmental Fate, Ecotoxicology, Residues, Metabolism and Toxicology) for the specific term ‘elemental iron’ performed in November 2019 (covering the period 2006-2017)

Stage of study selection process (for specific term ‘elemental iron’)	Number of summary records retrieved
Total number of summary records retrieved:	710
Number of summary records excluded from the search results after rapid assessment for relevance:	431
Total number of summary records remaining after filtering out those of clear irrelevance and manual de-duplication:	279
Total number of full-text documents assessed in detail:	37
Number of studies excluded from further consideration after detailed assessment for relevance and/or reliability:	31
Number of studies not excluded for relevance or reliability after detailed assessment:	5*
Number of studies not excluded for relevance or reliability after detailed assessment, that were relevant to the ecotoxicology assessment:	0

* Applicant’s text: “This figure is not “6”, as may be expected from taking into account the two rows directly above. This is because a single study not “excluded” after detailed assessment (Yameen *et al.*, 2013), and summarised in Appendix S3a, was nevertheless eventually awarded an evaluation indicating that it did not achieve a suitable standard of reliability (“Klimisch score 4 - not assignable”).”

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.9.1.1.3/1	Firman, J	1991	Nutrient requirements of Chickens and Turkeys	Y	N	n/a		N
B.9.1.1.3/1	Eissler, C. Firman, J.	1996	Effects of feather meal on the performance of Turkeys	Y	N	n/a		N
B.9.1.1.3/1	Dieumou, F. Adegbola, T. Doma, U.	2013	Growth Performance, carcass characteristics and economics of production of broilers fed diets with two sources of protein and two levels of wheat offal	Y	N	n/a		N
B.9.1.1.3/1	Moore, D., K. Baker, K. Thompson, E. Blair and J. Firman	2003	Digestible Sulfur Amino Acid Requirement of Male Turkeys During the 12 to 18 week Period.	Y	N	n/a		N
B.9.1.1.3/2	Ramsay, W. and Campbell, E.	1954	Iron metabolism in the Laying Hen	Y	N	n/a		N
B.9.1.1.3/3	Planas, J., De Castro, S. and Recio J. M.	1961	Serum Iron and its Transport Mechanism in the Fowl	Y	N	n/a		N
B.9.1.1.3/4	Dierenfield, E. M. Pinis, and C. Sheppard.	1995	Hemosiderosis and Dietary Iron in Birds	Y	N	n/a		N
B.9.1.1.3/5	Ramis, J. and Planas, J.	1978	Iron Metabolism in pigeons	Y	N	n/a		N
B.9.1.1.3/	Mete, A.,	2003	Iron	Y	N	n/a		N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
6	Hendricks, H., Klaren, P., Dorrestein, G., van Dijk, J., and Marx, J.		Metabolism in Mynah birds (<i>Gracula religiosa</i>) resembles human hereditary haemochromatosis (2003)					
B.9.1.2.2/1	LabDiet	None	Laboratory Rodent Diet 5001*	N	N	n/a		N
B.9.1.2.2/2	Reeves, P., Nielson, F., Fahey, G.	1993	AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet	N	N	n/a		N
B.9.3.1/1	Stanciu, O., L. Marghitas, D. Dezmirean and M. Campos	2011	A comparison between the mineral content of flower and honeybee collected pollen of selected plant origin (<i>Helianthus annuus</i> L. and <i>Salix</i> sp.). 2011.	N	N	n/a		N
B.9.6.2/1	Connorton, J., J. Balk, and J. Rodriguez-Celma	2017	Iron Homeostasis in Plants – a Brief Overview	N	N	n/a		N
B.9.6.2/2	Libralato, G., Costa Devoti, A.,	2015	Phytotoxicity of ionic, micro- and nano-sized	N	N	n/a		N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
	Zanella, M., Sabbioni, E., Micetic, I., Manodori, L., Pigozzo, A., Manenti, S., Groppi, F. and Volpi Ghirardini, A.		iron in three plant species					