Ferric pyrophosphate

Volume 3

Active substance data

Annex B.9: Ecotoxicology

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B.9 ECOTOXICOLOGY DATA

Introduction

BW01 GB is a non-volatile non-dusty molluscicide in the form of a granular bait, whose active substance is non-volatile, insoluble in water and organic solvents 3 % ferric pyrophosphate. It is intended for slug and snail control in all edible and inedible plants, open-grown and grown under protection. According to Good Agricultural Practice, the number of applications in a season is 1-6, and the maximum recommended dose is 50 kg of the product/ha (which equals 150 mg of ferric pyrophosphate per m2). Ferric pyrophosphate is an inorganic salt, whose components - iron and phosphorus - occur naturally in all ecosystems, both aquatic and terrestrial ones. These elements are nutrients which play a key role in the functioning of plants and animals. The amount of iron and phosphorus added to soil as a result of application compliant with GAP will be negligible compared to the natural concentration of these elements in soil. Ecotoxicological studies and toxicological assessment conducted indicate that ferric pyrophosphate used in the PPP does not pose a risk to non-target organisms.

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

Report: [redacted] 2013; Ferric Pyrophosphate: Avian Acute Oral Toxicity Study in Japanese Quail

Study code: P/13559/AAOT/13

Guideline: OECD Guideline No. 223

GLP: yes

Executive Summary:
In this study, single oral administration of the test article, Ferric Pyrophosphate, was made to a group of five birds at the limit dose of 2000 mg/kg body weight. A concurrent control group of five birds was treated with vehicle alone i.e. 1% Carboxy Methyl Cellulose. On the day of dosing all birds were observed continuously during the first two hours for regurgitation, onset of clinical signs and mortality, thereafter at 15 minutes, 30 minutes and 1, 2, 3, 4, 5, 6, 7 and 8 hours after dosing and thereafter they were observed once a day for 14 days. Their body weights were recorded at one day prior to dosing (day 0), on day of dosing (day 1), fasting body weight) and on days 3, 7, 14 and at test termination (day 15). The food intake was recorded daily until day 3, and then for the days 3 to 7 and days 7 to14 of the treatment. The test article did not induce any signs of toxicity in treated birds following dosing at 2000 mg/kg. Body weight gain by treated birds was found to be comparable to the control group birds. Also, no adverse effect on food consumption was observed in the treated birds as compared to the control group birds. Statistical estimate of the median lethal dose (LD_{50}) with 95% confidence intervals based on long term outcomes in female Japanese quail following acute oral exposure, was determined.
Estimated LD_{50} of Ferric Pyrophosphate is greater than 2000 mg/kg body weight.

I. MATERIALS AND METHODS

A. MATERIALS:

1.Test Material: Ferric Pyrophosphate
Description: Cream white powder
Batch No: 120327086
Purity: Assay: 101.73%
Storage: Room Temperature (27 ± 9 ºC)

2. Vehicle and/or positive control: 1% Carboxy Methyl Cellulose

3. Test animals
Species: Japanese Quail (*Coturnix japonica*)
Age: 6 weeks
Body weight: 187 – 226 g (Day 0)
Source: [redacted]
Diet: Commercial Quail ration supplied by Uttara Foods Pvt. Ltd., ad libitum
Acclimation period: 15 days in experimental room after veterinary examination

4. Environmental conditions
Temperature: 19 to 23 °C
Humidity: 63 to 66 %

Photoperiod: 8 hours light and 16 hours dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 21.06.2013 – 20.07.2013

2.Experimental treatments

The limit dose test design consists of dosing five birds simultaneously at the limit dose in addition to sham dosing in the control birds. Birds were observed for 15 days. Formulation of the test article for dosing was freshly prepared before dosing. The test article was uniformly suspended in 1.0 % Carboxy Methyl Cellulose to obtain a final concentration of 200 mg/ml. The dose administered to individual bird was adjusted according to its body weight that was recorded just before dosing to give a constant dosage volume of 10 ml/kg body weight. Control group birds were administered vehicle alone i.e. 1.0 % Carboxy Methyl Cellulose, at the same dosage rate of 10 ml/kg body weight.

3.Observations

The following observations were made during the test:
- mortality
- clinical signs
- body weights
- food consumption
- necropsy
- LD50 calculation

4. Data analysis

The LD50 value could not be calculated as no mortality was observed at the limit dose of 2000 mg/kg body weight. As per the recommendation of guideline OECD 223, SEDEC Programme was used for the study.

5.Deviations to the Guideline

There were no deviations from the Guideline.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.1-1 Summary of body weights – female birds

Dose Group and dose (mg/kg)		Day							
		Day 0	Day 1 (Fasting Body weight)	Day 3	Day 7	% Gain (0-7)	Day 14	Day 15	% Gain (0-15)
G1 0 (Vehicle control 1.0% Carboxy Methyl Cellulose)	Mean	195.80	186.20	199.60	206.00	5.23	217.60	218.60	11.63
	±S. D	10.43	9.31	10.64	10.22	-	11.82	12.20	-
G2 2000	Mean	207.60	197.40	211.20	216.20	4.15	230.40	230.60	11.11
	±S. D	15.90	15.04	16.21	16.42	-	16.52	16.82	-

Table 9.1-2 Summary of food consumption (g/bird/day) – female birds

Dose Group and dose (mg/kg	Food consumption	Day					Average
		0	2	3	7	14	
G1 0 (Vehicle Control i.e. 1.0% Carboxy methyl cellulose)	Average	23,8	24,6	25,6	31,4	29,5	26,98

G2 2000	Average	23,8	24,4	25,2	31,0	29,8	26,84
	% of control	100	99	98	99	101	99

B. OBSERVATIONS

No mortality was observed in treated and control group birds throughout the 15 days observation period. No clinical signs were observed in treated and control group birds throughout 15 days observation period. Body weight gain by treated birds at the dose of 2000 mg/kg was not affected and was found comparable to the vehicle control group birds. No effect on food intake was observed in the treated birds at the dose of 2000 mg/kg as compared to the vehicle control group birds.

III. CONCLUSIONS

Estimated LD_{50} of Ferric Pyrophosphate is greater than 2000 mg/kg body weight.

RMS comment: The study performed in GLP conditions and according to OECD guideline No. 223 is considered acceptable.The estimated acute oral LD_{50}> 2000 mg/kg bwfor female Japanese Quail.
The result (endpoint) may be used for risk assessment

B.9.1.1.2 Short-term dietary toxicity to birds

The results of studies conducted on mammals indicate that there is no health risk resulting from the possibility of exposure by oral route, in both acute dietary toxicity study (see point B 9.1.2.1) and in short-term toxicity study (see points B 6.3.1 and B 6.3.2), thus data on short-term dietary toxicity to birds is not required.

B.9.1.1.3 Sub-chronic toxicity and reproduction to birds

Report: [redacted]2015, Ferric Pyrophosphate Avian Reproduction Test (*Coturnixcoturnix japonica*)
Study code: G/33/14
Guideline: OECD Guideline No. 206 (1984)
GLP: YES
Executive Summary:
The reproduction study of Ferric Pyrophosphate was performed on the Japanese quail (*Coturnixcoturnix japonica*). The birds were 6 weeks old. The exposure lasted 20 weeks. During this period, the birds received fodder containing the test item at the concentrations of 40, 200 and 1000 mg/kg fodder. At the end of the first exposure period (ten weeks), the birds were induced, by photoperiod manipulation, to lay eggs. Eggs were collected over a 10-week period, incubated, and hatched. The young were maintained for 14 days. The hatching process was repeated five times.
During the study, toxic effects of the test item, food consumption, body weights of adult birds, average body weights of young birds, egg production, eggshell thickness, viability, hatchability, and survival were determined. Gross examinations of all adult birds were also performed. The test item at the concentrations of 40, 200, and 1000 mg/kg fodder caused no mortality of adult birds. After application of the test item, no clinical symptoms were observed. On the basis of the gross examinations, it may be concluded that 20-week oral administration of the test item at the concentrations mentioned above did not lead to any macroscopic changes (70 out of 72 birds exposed to the test item had no pathological changes). The test item had no influence on fodder consumption. Food consumption by both adult and young birds from all groups was similar. The test item had no impact on body weights of both adult and young birds. Mean body weights during the experiment were similar. The test item had no influence on the evaluated reproduction parameters. No statistically significant differences in the total numbers of laid eggs, incubated eggs, living embryos, hatchlings, and 14-day-old birds and eggshell thickness between the control and the treated groups were observed. The concentration of the test item in a diet which results in a 20% reduction in reproduction of birds (EC_{20}) is higher than 1000 mg/kg fodder. The concentration of the test item in a diet which results in a 10% reduction in reproduction of birds (EC_{10}) is higher than 1000 mg/kg fodder. The test item, Ferric Pyrophosphate at the concentrations of 40, 200, and 1000 mg/kg fodder did not have a significant effect on reproduction of the Japanese quails. The maximum concentration at which the test item is observed to have no negative effects on reproduction (NOEC) is ≥ 1000 mg/kg fodder (i.e. ≥ 111.2 mg/kg b.w./day).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Ferric Pyrophosphate

Description: cream powder
Batch No: 120327086
Purity: 101.73%
Storage: cool and dry place

2. Vehicle and/or positive control: fodder

3. Test animals

Species: the Japanese quail, *Coturnixcoturnix japonica*.
Age: 10 days (preliminary experiment) 6 weeks (main experiment)
Sex: Males and females
Source: [redacted]
Diet: mixed food for adult birds and food for young birds from UNIPASZ Sp. J. Siemiatycze, water ad libitum
Acclimation period: 14 days

Environmental conditions for adult birds

Temperature: 20 – 29°C
Humidity: 40 – 85%
Photoperiod: 8 h light/16 h dark during the first eight weeks of the test (stage I); 16 – 18 h light/8 – 6 h dark during the laying period (stage II)
Air exchange: no data

Environmental conditions for young birds from the F1 generation:

Temperature: 34 - 40°C during the first week of life; 28 – 32°C during the second week of life
Humidity: 55 – 74% during the first week of life; 50 – 68% during the second week of life
Photoperiod: 24 h light during the first week of life; 14 h light/10 h dark during the second week of life.
Air exchange: no data

B. STUDY DESIGN AND METHODS:

1. In life dates: 27/06/14 – 16/12/14

2. Experimental treatments:

Adult birds were fed with a diet containing the test item for a period of 20 weeks (10 weeks before egg laying). Eggs were collected over a ten-week period and hatched. The birds were randomly distributed to the treated and the control groups. Each group consisted of 24 birds (12 roosters and 12 hens). Twelve cages were used. There were 1 rooster and 1 hen in each cage. The concurrent control group was fed with food without the test item. A label specifying the study code, the concentration of the test item, the cage number, and the date of the beginning of the test was attached to each cage. During the test, ad libitum access to drinking water was provided. The individual groups of birds received food with the test item at three concentrations for 20 weeks (10 weeks before egg laying). Food was replaced with a fresh portion once a week. At the end of the first stage of the test, the birds were induced, by photoperiod manipulation, to lay eggs. Eggs were collected every day over a ten-week period. Damaged eggs were removed. Each egg was marked with the cage number, stored in a thermostatic cabinet, candled to detect cracks, artificially incubated for 14 days, and hatched. Eggs were candled again between day 6 and day 11 of incubation in order to determine viability. After 14 days, all young birds were euthanized. During the test, all eggs laid on days 5, 20, 35, 50, and 65 were measured for eggshell thickness five times.

3. Observations:

The following observations were made during the test:

- mortality and signs of toxicity;
- body weights of adults at the beginning of the exposure period, prior to the onset of egg laying, and at the end of the test;
- body weights of young, i.e. 14-day-old birds;
- food consumption by adults after each week;
- food consumption by young birds: the first and the second weeks after hatching;
- general gross examinations of all adult birds;
- the total number of eggs;

- the number of incubated eggs, i.e. the egg set (without eggs with cracks and the ones removed for eggshell thickness measurement);
- viability;
- the number of young birds;
- the number of 14-day-old birds;
- eggshell thickness.

4. Results analysis

The results were analyzed to determine the impact of the test item on reproduction of the Japanese quail, Coturnixcoturnix japonica and to calculate the NOEC (no observed effect concentration), the maximum treatment level used in a test which produces no adverse effects on the number of eggs, the set of eggs, viability, hatchability, the number of survivors and eggshell thickness. The NOEC was expressed in mg test item per kg food and converted into mg test item per kg b.w. per day.

In order to determine the NOEC, the following statistical tests were used:

- Shapiro-Wilk's test on normal distribution,
- Levene's Test on variance homogeneity,
- Dunnett's multiple t-test – comparison of the treated groups to the control one in order to determine statistically significant differences (significance level, alpha = 0.05).

Moreover, the following values were calculated:

- the EC_{20} (effect concentration), i.e. the concentration of the test item in a diet which results in a 20% reduction in reproduction of birds;
- the EC_{10} (effect concentration), i.e. the concentration of the test item in a diet which results in a 10% reduction in reproduction of birds.

The EC_{20} and the EC_{10} were determined using a probit method.

To make calculations, the ToxRat Professional 2.10 computer software was used.

5. Deviations

The following deviations from the Study Plan occurred:

1. Temperature for adult birds was between 20 - 29°C. According to the Study Plan, it should have ranged from 17 to 27°C. However, it was a short-term deviation which did not affect the course of the experiment or the results.
2. Air humidity was between 40 – 85%. According to the Study Plan, it should have ranged from 50 to 75%. However, it was a short-term deviation which did not affect the course of the experiment or the results.
3. Temperature during the first week of rearing was between 34 – 40°C. According to the Study Plan, it should have ranged from 35 to 38°C. However, it was a short-term deviation which did not affect the course of the experiment or the results.
4. Temperature during the second week of rearing was between 28 – 32°C. According to the Study Plan, it should have ranged from 30 to 32°C. However, it was a short-term deviation which did not affect the course of the experiment or the results.
5. The ranges of temperature and air humidity during egg storage were 14.5 – 16.5°C and 50 – 83% (45 % for a few hours), respectively. According to the Study Plan, they should have been 15.0 – 16.0°C and 55 – 75%, respectively. However, these were short-term deviations which did not affect the course of the experiment or the results.
6. The ranges of temperature and air humidity during egg incubation were 37.1 – 37.2°C and 50 – 73%, respectively. According to the Study Plan, they should have been 37.5°C and 50 – 70%, respectively. However, these were short-term deviations which did not affect the course of the experiment or the results.
7. The ranges of temperature and air humidity during hatching were 37.2 – 37.5°C and 60 –78%, respectively. According to the Study Plan, they should have been 37.5°C and 70 – 75%, respectively. However, these were short-term deviations which did not affect the course of the experiment or the results.

Decreases of temperature and humidity were caused by opening of the incubator.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.1-3 Summary of test results

Ferric pyrophosphate [mg a.s./kg feed]	Control	40	200	1000
Reproductive performance				
Average number of laid eggs per hen	60,7	63,8	61,5	58,8
Average number of eggs in a set	55,3	58,2	56,3	53,7

Average number of living embryos		47,8	53,8	50,7	47,1
Average number of hatchlings		41.9	47.5	43.3	41.4
Average number of 14–day-old birds per hen		40.4	44.0	40.6	39.2
Fertilization [%]		86.4	92.4	90.0	87.7
Hatching [%]		87.7	88.3	85.4	87.9
% of 14-day- old birds		96.4	92.6	93.8	94.7
Eggshell thickness					
Mean shell thickness [mm]		0.25	0.25	0.26	0.25
Body weight of 14-day old survivors					
Mean body weight [g]		40.5	41.3	41.4	40.8
Average body weight [g]					
Test initiation	M	162.8	170.0	160.5	168.5
	F	205.7	203.8	203.6	201.3
Test termination	M	208.0	214.3	210.9	217.6
	F	254.3	250.7	257.2	250.1
Average feed consumption [g/bird/week]					
Week 1	M + F	170.9	158.0	159.4	167.5
Week 5	M + F	157.8	162.2	161.4	153.3
Week 10	M + F	171.1	171.2	180.6	168.8
Week 15	M + F	184.3	183.7	188.9	186.2
Week 20	M + F	182.4	182.1	183.0	180.6

Table 9.1-4 Endpoints

Parameter	EC_{20}	EC_{10}	NOEC
Number of laid eggs	> 1000	> 1000	≥ 1000
Number of eggs in a set	> 1000	> 1000	≥ 1000
Number of living embryos	> 1000	> 1000	≥ 1000
Number of hatchlings	> 1000	> 1000	≥ 1000
Number of 14-day-old birds	> 1000	> 1000	≥ 1000
Eggshell thickness	> 1000	> 1000	≥ 1000

NOEC ≥ 1000 mg/kg food corresponds to ≥ 111.2 mg/kg b.w./day

B. OBSERVATIONS

The test item at the concentrations of 40, 200, and 1000 mg/kg fodder caused no mortality of adult birds. After application of the test item, no clinical symptoms were observed. On the basis of the gross examinations, it may be concluded that 20-week oral administration of the test item at the concentrations mentioned above did not lead to any macroscopic changes (70 out of 72 birds exposed to the test item had no pathological changes). The test item had no influence on fodder consumption. Food consumption by both adult and young birds from all groups was similar. The test item had no impact on body weights of both adult and young birds. Mean body weights during the experiment were similar. The test item had no influence on the evaluated reproduction parameters. No statistically significant differences in the total numbers of laid eggs, incubated eggs, living embryos, hatchlings, and 14-day-old birds and eggshell thickness between the control and the treated groups were observed. The concentration of the test item in a diet which results in a 20% reduction in reproduction of birds (EC_{20}) is higher than 1000 mg/kg fodder. The concentration of the test item in a diet which results in a 10% reduction in reproduction of birds (EC_{10}) is higher than 1000 mg/kg fodder.

III. CONCLUSIONS

The test item, Ferric Pyrophosphate at the concentrations of 40, 200, and 1000 mg/kg fodder did not have a significant effect on reproduction of the Japanese quails. The NOEC (no observed effect concentration), i.e. the

maximum treatment level used in a test which produces no adverse effects on the parameters of reproduction evaluated during the experiment is ≥ 1000 mg/kg fodder (i.e. ≥ 111.2 mg/kg b.w./day).

RMS comment: The study of sub-chronic and reproduction toxicity to birds performed in GLP conditions and according to OECD guideline No. 206 is considered acceptable.The estimated NOEC≥1000 mg/kg fodder (i.e. ≥ 111.2 mga.s. /kg b.w./day) for the Japanese quail (*Coturnixcoturnix japonica*). The result may be used for risk assessment.

B.9.1.2 Effects on terrestrial vertebrates other than birds

B.9.1.2.1Acute oral toxicity to mammals

Report: [redacted]2013 FERRIC PYROPHOSPHATE: Acute Oral Toxicity Study on Rats – Fixed Dose Method
Study code: PO-2/13
Guideline: OECD Guideline No. 420
GLP: yes

Executive Summary:
The acute oral toxicity study – fixed dose method was performed in order to obtain information on health hazards likely to arise from a single oral exposure caused by the consumption of the test item, ferric pyrophosphate. The experiment commenced with a sighting study in which one animal received the test item at a dose of 300 mg/kg b.w. Since this dose did not cause any toxicity signs or mortality, the test item at a dose of 2000 mg/kg b.w was administered once to the next female. On the grounds of the results of the sighting study, four animals in the main study were given the test item at a dose of 2000 mg/kg b.w. (the animal used in the sighting study was included in the main study). Before the administration,the animals had been fasted for about 18-19 hours. The test item in the form of a suspension in 0.5% carboxymethylcellulose in a volume of 0.5 mL/100 g b.w. was administered using a metal stomach tube. After the administration of the test item, the animals were observed for 14 days. General and detailed clinical observations were conducted daily during the entire experiment. Body weights of the animals were determined on days 0 (directly before the administration of the test item), 7, and 14. After a 14-day observation period, all animals which survived the experiment were euthanized and subjected to a necropsy and a detailed gross examination.
Following a single administration of the test item at a dose of 300 mg/kg b.w. to one animal used in the sighting study, the animal did not exhibit any clinical signs during the whole observation period. The animal survived the 14-day observation period.
Following a single administration of the test item at a dose of 2000 mg/kg b.w. to one animal used in the sighting study, the animal did not exhibit any clinical signs during the whole observation period. The animal survived the 14-day observation period.
Following a single administration of the test item at a dose of 2000 mg/kg b.w. to the next four animals used in the main study, the animals did not exhibit any clinical signs during the whole observation period. The animals survived the 14-day observation period. In all animals which survived the experiment, body weight gain was stated. The gross examinations of the animals did not reveal any pathological changes.
On the grounds of the study results, the test item, i.e. ferric pyrophosphate can be classified to the following categories:
- category 5/beyond classification – according to the Globally Harmonized System (GHS),
- beyond categorization – according to the Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of December 16th, 2008 on classification, labelling, and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC and amending Regulation (EC) No. 1907/2006.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Ferric pyrophosphate
Description: cream powder
Batch No: 120327086
Purity: 101.73%
Storage: at temperature between 10-30 ºC and without access to water

2. Vehicle and/or positive control: 0.5% carboxymethylcellulose

3. Test animals
Species: Wistar female rats

Age: 9 - 11 weeks
Weight: 204, 207 (sighting study), about 197 (main study)
Source: [redacted]
Acclimation period: 5 days

Environmental conditions
Temperature: 19 – 24 °C
Humidity: 5 – 60%
Photoperiod: 12 hours light / 12 hours dark
Air exchange: about 16 times / hour

B. STUDY DESIGN AND METHODS:

1. In life dates: 19.02.2013 - 20.03.2013

2. Experimental treatments

Sighting study

The test item at a dose of 300 mg/kg b.w. was administered to one animal. This dose was chosen on the basis of the available literature data on the active substance contained in the test item. The test item was administered in the form of a suspension in 0.5% carboxymethylcellulose in a volume of 0.5 mL/100 g b.w. using a metal stomach tube. One milliliter of the administered suspension contained 60 mg of the test item. The suspension was prepared on the administration day shortly before the administration. Since the dose of 300 mg/kg b.w. did not cause any clinical signs or mortality, the next female received the test item at a dose of 2000 mg/kg b.w. The test item was administered in the form of a suspension in 0.5% carboxymethylcellulose in a volume of 0.5 mL/100 g b.w. using a metal stomach tube. One milliliter of the administered suspension contained 400 mg of the test item. The suspension was prepared on the administration day shortly before the administration.

Main study

The test item at a dose of 2000 mg/kg b.w. was administered to four animals. The animal from the sighting study which received the same dose was included in the main study. The test item was administered in the form of a suspension in 0.5% carboxymethylcellulose in a volume of 0.5 mL/100 g b.w. using a metal stomach tube. One milliliter of the administered suspension contained 400 mg of the test item. The suspension was prepared on the administration day shortly before the administration.

3. Observations

The evaluation of general condition of the animals, i.e. the observation of all animals for morbidity and mortality was conducted twice a day or once a day (on days off) during the 14-day observation period. The detailed clinical observations were performed on the administration (day 0) 10, 30, and 60 minutes after the administration and then at hourly intervals up to the 5th hour after the administration. From the 1st to the 14th day of the observation period, the detailed clinical observations were performed once a day.

Body weights of the animals were determined directly before the administration of the test item (day 0) and then on the 7th and 14th day (before euthanasia).

The detailed gross examinations focused on the external body surfaces, all openings, and the cranial, thoracic, and abdominal cavities with their content.

4. Results analysis: no data

5. Deviations

During the experiment, the relative air humidity was lower than 30% a few times. These changes did not influence the results of the experiment. No other deviations from the Study Plan were stated.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.1-5 Body weight of the animals (g) – overalllist

Dose (mg/kg b.w.)	Animal no.	Day of experiment / Body weight (g)			Body weight gain (g) (0→14)
		0	7	14	
300	1*	204	239	255	51
2000	1*	207	214	229	22
	2	200	226	232	32
	3	196	231	238	42
	4	199	230	235	36
	5	191	219	239	48

* the female from the sighting study

B. OBSERVATIONS

Sighting study

Following a single administration of the test item at a dose of 300 mg/kg b.w. to one animal and 2000 mg/kg b.w. to other animal used in the sighting study, the animal did not exhibit any clinical signs during the whole observation period. Both animals survived the 14-day observation period. In both animals, body weight gain was stated during the whole experiment. The gross examination did not reveal any pathological changes.

Main study

Following a single administration of the test item at a dose of 2000 mg/kg b.w. to the next four animals used in the main study, the animals did not exhibit any clinical signs during the whole observation period. The animals survived the 14-day observation period. In all animals, body weight gain was stated during the whole experiment. The gross examination did not reveal any pathological changes.

III. CONCLUSIONS

On the grounds of the study results, the test item, ferric pyrophosphate can be classified to the following categories:

- category 5/ beyond classification – according to the Globally Harmonized System (GHS),
- beyond categorization – according to the Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of December 16th, 2008 on classification, labelling, and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC and amending Regulation (EC) No. 1907/2006.

RMS comment: The study performed in GLP conditions and according to OECD guideline No. 420 is considered acceptable. The estimated acute oral LD_{50} for female rats is above 2000 mg/kg bw.
The result (endpoint) may be used for risk assessment.

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

The conducted study of sub-chronic and reproductive toxicity to birds (see point B9.1.1.3) demonstrated a lack of health risk resulting from oral exposure to ferric pyrophosphate, thus a study of long-term and reproductive toxicity to mammals was not conducted. Based on the natural occurrence of iron and phosphorus in the environment, and the use of ferric pyrophosphate as a dietary supplement and food additive, harmful effect of the substance on reproduction and long-term toxicity can be ruled out.

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

Ferric pyrophosphate is insoluble in organic solvents, thus its bioconcentration in organisms is not expected. Moreover, iron and phosphorus are elements naturally occurring in all organisms, they are indispensable for their proper functioning and for metabolic processes, and their amount absorbed from food is strictly regulated.

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

Based on the conducted studies of acute and long-term toxicity to birds, as well as literature data on the use of ferric pyrophosphate as a dietary supplement and food additive, and natural occurrence of iron and phosphorus in the environment, it is believed that ferric pyrophosphate does not pose a risk resulting from oral exposure and does not demonstrate any toxic properties towards wild land vertebrates.

B.9.1.5 Potential for endocrine disruption

Ferric pyrophosphate is a substance commonly considered safe and approved for use as a dietary supplement and food additive, thus negative impact on the endocrine system is not expected.

Information on potential endocrine disrupting properties for non-mammals of Ferric pyrophosphate was retrieved from the studies on Sub-chronic and reproductive toxicity to birds (OECD TG 206), described in detail in point B. 9.1.1.3. The OECD Revised (2018) Technical Guidance Document No 150 postulates that, although it was not validated yet, some of the endpoints in this apical test are nevertheless potentially affected by oestrogen, androgen, thyroid, steroidogenesis (E,A,T,S) EDs. Of particular interest in the context of oestrogens, androgens and steroidogenesis disrupters are egg production, embryo viability and hatchability.

Avian Reproduction Test

Report: [redacted] 2015, Ferric Pyrophosphate Avian Reproduction Test (*Coturnix coturnix japonica*)

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Ferric Pyrophosphate - detailed description of the test material is contained in point B.9.1.1.3

2. Vehicle and/or positive control: fodder

3. Test animals: the Japanese quail, Coturnix coturnix japonica - detailed description of the test animals and their housing conditions and diet is contained point B.9.1.1.3

B. STUDY DESIGN AND METHODS:

The full description of the study design and methods is given in point B.9.1.1.3. Only information important for assessment of endocrine disrupting properties is given below.

Adult birds (6 weeks old at the beginning of the experiment) were fed with a diet containing the test item in concentrations of 40, 200 and 1000 mg/kg of the fodder (the highest concentration foreseen in this test) for a period of 20 weeks. Considering the food consumption the diet of 1000 mg/kg fodder corresponds to administration of 111.2 mg/kg body weight per day. The control group was fed with the fodder without the test compound. There were 12 females and 12 males in every dosed and in the control group. The birds were housed in cages – one hen and one rooster in a cage.

At the end of the first stage of the test, i.e. after 10 weeks of dosing, the birds were induced, by photoperiod manipulation, to lay eggs. The process was repeated five times in a period of the second ten weeks of the experiment. Eggs were collected every day over a whole ten-week period. Damaged eggs were removed. Each egg was marked with the cage number, stored in a thermostatic cabinet, candled to detect cracks, artificially incubated for 14 days, and hatched. Eggs were candled again between day 6 and day 11 of incubation in order to determine viability. After 14 days, all young birds were euthanized. During the test, all eggs laid on days 5, 20, 35, 50, and 65 of the second ten-week period were measured for eggshell thickness.

II. RESULTS

The test item at the concentrations of 40, 200, and 1000 mg/kg fodder caused no mortality of adult birds. After application of the test item, no clinical symptoms were observed. On the basis of the gross examination, it may be concluded that 20-week oral administration of the test item at the concentrations mentioned above did not lead to any macroscopic changes (70 out of 72 birds exposed to the test item had no pathological changes). The test item had no influence on fodder consumption. Food consumption by both adult and young birds from all groups was similar. The test item had no impact on body weights of both adult and young birds. Mean body weights during the experiment were similar.

The test item had no influence on the evaluated reproduction parameters. No statistically significant differences in the total numbers of laid eggs, incubated eggs, living embryos, hatchlings, and 14-day-old birds and eggshell thickness between the control and the treated groups were observed. Detailed information on the results may be found in point B.9.1.1.3.

Table 1. Summary of test results

Ferric pyrophosphate [mg/kg fodder]	**Control**	**40**	**200**	**1000**
Reproductive performance				
Average number of laid eggs per hen	60,7	63,8	61,5	53,8
Average number of eggs in a set	55,3	58,2	56,3	53,7
Average number of living embryos	47,8	53,8	50,7	47,1
Average number of hatchlings	41,9	47,5	43,3	41,4
Average number of 14-day-old birds per hen	40,4	44,0	40,6	39,2

Fertilization [%]	86,4	92,4	90,0	87,7
Hatching [%]	87,7	88,3	85,4	87,9
% of 14-day-old birds	96,4	92,6	93,8	94,7
Eggshell thickness				
Mean shell thickness [mm]	0,25	0,24	0,26	0,25
Body weight of 14-day-old survivors				
Mean body weight [g]	40,5	41,3	41,4	40,8

III. DISCUSSION AND CONCLUSIONS

The test item, Ferric pyrophosphate, at the concentrations of 40, 200, and 1000 mg/kg fodder did not have a significant effect on reproduction of the Japanese quails. Egg production, embryo viability and hatchability were not affected by the compound. Also other endpoints which may be responsive to some endocrine disrupters, e.g. growth which may respond to some thyroid disrupters or percentage of cracked eggs and shell thickness which may respond to chemicals interfering with the control of shell deposition, were not affected. The endocrine disrupting properties were not demonstrated in the study.

On the basis of this study and taking into account that no endocrine properties were revealed in studies on other vertebrates (the Repeated Dose 28-Day Oral Toxicity Study in Rodents and the Repeated Dose 90-Day Oral Toxicity Study in Rodents) it may be concluded that there is no concern for endocrine disrupting potential of Ferric pyrophosphate for terrestrial vertebrates.

According to Commission Regulation (EU) 2018/605 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties Ferric pyrophosphate, considering non-mammals, should be classified as not having endocrine disrupting properties.

RMS: According to the ED criteria[1], a substance shall be considered as having ED properties if it meets all of the following criteria:
a) it shows an adverse effect in non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub) that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
b) it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;
c) the adverse effect is a consequence of the endocrine mode of action.

In the study of [redacted] (2015) Ferric Pyrophosphate Avian Reproduction Test (Coturnix coturnix japonica) the adverse effects, including effects on reproduction, were not seen in Japanese quail exposed to Ferric Pyrophosphate in a diet/fodder at the maximal dose of 111.2 mg/kg bw/day. The study has been performed according to OECD TG 206 and includes several parameters which are relevant for identification of ED properties of the substance.
Avian Reproduction Test (OECD TG 206) is a method belonging to level 4 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals, revised 2017, including In vivo assays providing data on adverse effects on endocrine-relevant endpoints. The mode of action which may produce a response in this test would be EAST endocrine modalities. EATS modalities can affect: egg production, cracked eggs, eggshell thickness, egg viability, hatchability, body weight, gross pathology. The lack of significant effect on reproduction of the Japanese quails, egg production, embryo viability and hatchability, as well as growth of birds or percentage of cracked eggs and shell thickness, shows that the first criterion, i.e. the adverse effect in non-target organism/birds, was not fulfilled and that ferric pyrophosphate should not be considered as having endocrine disrupting properties for birds/non-target organisms. Studies on the mode of action are not necessary.

[1] Commission Regulation (EU) 2018/605 of 19 April 2018 setting out scientific criteria for the determination of endocrine disrupting and amending Annex II to Regulation (EC) 1107/2009. OJ L 101, 20.4.2018, p. 33–36.
Available online: http://data.europa.eu/eli/reg/2018/605/oj

B.9.2 Effects on Aquatic Organisms

B.9.2.1 Acute toxicity to fish

Report: [redacted] 2013; Fish Acute Toxicity Test according to OECD Guideline No 203
Study code: 0003/0024/E
Guideline: OECD 203
GLP: yes
Executive Summary:
The acute toxicity study of the test item, iron pyrophosphate, for rainbow trout (*Oncorhynchus mykiss*) was conducted. The aim of the study was to determine LC_{50}, LC_{20} and LC_{10} values calculated on the basis of observed fish mortality symptoms after 24, 48, 72 and 96 hours of exposure. The iron pyrophosphate has been non-toxic in the highest measured test item concentration 134 µg/l, being it's solubility limit, and corresponding to nominal concentration of 100 mg/l in this limit test. 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. Experiment with reference substance - potassium dichromate ($K_2Cr_2O_7$) was performed. Basing on the actual observations value NOEC ≥ 134 µg/l was estimated for determined concentration and NOEC ≥ 100 mg/l for nominal concentration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Iron pyrophosphate
Description: cream-colored powder
Batch No: 120327086
Purity: not lower than 98%
Storage: cool and dry place

2. Vehicle and/or positive control: tap water: total hardness 14 0 N / potassium dichromate ($K_2Cr_2O_7$)

3. Test animals
Species: rainbow trout (*Oncorhynchus mykiss*)
Weight: 2,5 – 3,0 g
Source: [redacted]
Diet: standard granulated fish food produced by ALLER AQUA,
Acclimation period: one month

4. Environmental conditions
Temperature: 14,40°C - 16,10 °C
Carbonate hardness: 250 mg/l
pH: 8,5
Photoperiod: fluorescent lighting, 16 h day: 8 h night
Oxygen concentration: not lower than 60%

B. STUDY DESIGN AND METHODS:

1. In life dates: started on 24th June 2013, ended on 28th June 2013

2. Experimental treatments

Preliminary test

In the preliminary test the following concentrations of test item were used: 16,75; 33,5; 67 and 134 µg/l. As test vessels 10 liter glass aquariums were used. Aquariums were cover in order to prevent the loss of fish (jumping out), reduce evaporation as well as reduce the risk of accidental pollution. Each concentration was prepared in a single replicate, and into each of them, 3 fish were introduced. The feeding of fish was suspended 24 hours before the beginning of the experience. The experiment was carried out in a static system and lasted for four days. Due to the poor solubility of the test item in a medium, a stock solution with 100 mg of test item added per 1 litre was prepared by stirring the test item in the medium for 72 hours using a mechanical stirrer. Afterwards, the solution was filtered through a membrane filter with a pore size of 0,45 µm. Further concentration of the test material were obtained by previous concentration.

Definitive test

The test was conducted in the continuously aerated, 20 liter glass aquariums. Aquariums were cover in order to prevent the loss of fish (jumping out), reduce evaporation as well as reduce the risk of accidental pollution. The test lasted 96 hours. The test was prepared in a single replicate with seven fish introduced into each aquarium.

The feeding of fish was suspended 24 hours before the beginning of the experiment. Based on the results from stability test, the definitive experiment was conducted in static exposure system, the solution was not replaced by a fresh one.

Reference test

Experiment with reference substance - potassium dichromate ($K_2Cr_2O_7$) was performed. The following concentrations were provided into the medium: 32 mg/l, 56 mg/l, 100 mg/l, 180 mg/l and 320 mg/l. The test was prepared with seven fish introduced into each aquarium. The experiment lasted 96 h and was carried out in static exposure system. Dead fish were removed from aquariums. The same test conditions and medium like in the definitive test were used.

3. Observations

During the experiment, the water temperature was recorded (additional vessel was positioned directly next to the test vessels). On the start of the experiment and at the end, as well as after every 24 hour of the study, the water parameters - pH and dissolved oxygen values of control and test item concentration were measured. Observations of fish mortality and intoxication symptoms e.g.: loss of balance, change in pigmentation, lethargy, swimming at the surface, swimming at the bottom, hyperactivity, hyperventilation, agony or swollen abdomen in each aquarium after 3h, 6h, 24h, 48h, 72h and 96h (± 1 h) after the start of the experiment were made.

4. Statistical calculations

On the basis of the obtained data the calculations were made according to OECD Guideline No 203.

LC_{50}, LC_{20} and LC_{10} for the fish mortality along with NOEC and LOEC values were calculated using a statistical program - ToxRat Professional.

ToxRat Professional used the following statistical analyses for the calculation:

- probit analysis using linear max. likelihood regression
- Fisher's exact binomial test with Bonferroni correction

5. Deviations to the Guideline

There were no deviations from the Guideline.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.2-1 Fish mortality in the preliminary test – raw data

Concentration of Test item [µg/l]	24 hours		48 hours		72 hours		96 hours	
	Mortality during the readout	Total mortality	Mortality during the readout	Total mortality	Mortality during the readout	Total mortality	Mortality during the readout	Total mortality
Control	0	0	0	0	0	0	0	0
16,75	0	0	0	0	0	0	0	0
33,5	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0
134	0	0	0	0	0	0	0	0

Table 9.2-2 Fish mortality in the definitive test – raw data

Time	Concentration of test item [µg/l]	Mortality during the readout	Total mortality
24 h	control	0	0
	134	0	0
48 h	control	0	0
	134	0	0
72 h	control	0	0
	134	0	0
96 h	control	0	0
	134	0	0

Table 9.2-3 Fish (control population) bodyweight and body length

No.	Control		Concentration of test item 134 µg/l	
	Weight [g]	Length [cm]	Weight [g]	Length [cm]
1	2,22	5,9	2,35	5,8
2	2,26	5,9	2,55	5,9
3	2,92	6	2,20	5,9
4	2,57	6	2,75	6
5	1,74	5,5	2,85	5,9
6	2,80	5,9	2,52	5,8
7	2,92	6,0	2,89	6
Mean	2,49	5,89	2,59	5,9
SD	0,44	0,18	0,26	0,08

B. OBSERVATIONS

In none of the tested concentrations fish mortality or signs of intoxication were observed. On the basis of these results during the definitive test, the limit test was performed using single concentration of the test substance - 134 µg/l which is the limit of its solubility.

III. CONCLUSIONS

The test material is non-toxic in the determined test item concentration 134 µg/l, being it's solubility limit, andcorresponding 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 NOEC ≥ 134 µg/l was estimated for determined concentration and NOEC ≥ 100 mg/l for nominal concentration.

RMS comments:

The study performed in GLP conditions and according OECD TG 203 is considered valid and established endpoints may be used for risk assessment, however with considerable uncertainty since no mortality or any toxic effect were observed at the highest concentration used in the test. No significant deviations from the test guideline were noted. All validity criteria were met. The definitive test was performed with saturated solution of the test item with concentration of the test item measured to be 134 µg/l in the nominal stock solution containing 100 mg of iron pyrophosphate in 1 L litre of medium.

The study is considered acceptable with following endpoints:

96h LC_{50} is greater than 134 µg a.s./L, which a limit of solubility of iron pyrophosphate in the stock solution containing 100 mg of iron pyrophosphate in 1 L litre of medium, thus nominally greater than 100mg/L.
Since no signs of intoxication of fish were observed during 96h test a NOEC (no observed effect concentration) of iron pyrophosphate in these test conditions amounts to ≥ 134 µg/l being a solubility limit of iron pyrophosphate in the solution containing nominally 100 mg of iron pyrophosphate in 1 L litre of medium.

B.9.2.2 Long-term and chronic toxicity to fish

B.9.2.2.1 Fish early life stage toxicity test

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 (see point B.9.2.1) , based on the EU Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4) the Fish Early Life Stage (FELS) of Zebrafish (*Danio rerio*) test was conducted for the BW01 GB formulation.

B.9.2.2.2 Fish full life cycle test

Since the Fish early life stage (FELS) test conducted for the BW01 GB formulation demonstrated that the material studied is not toxic to *Danio rerio* in the early developmental stages and since ferric pyrophosphate does not bioconcentrate, based on the EU Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4) it is believed that a fish full life cycle study is not necessary.

B.9.2.2.3 Bioconcentration in fish

Ferric pyrophosphate is insoluble in organic solvents, thus its bioconcentration in fish is not expected. Moreover, iron and phosphorus are elements naturally occurring in both the aquatic ecosystem and all living organisms, they are indispensable for their proper functioning and for metabolic processes, and their amount absorbed from food is strictly regulated.

B.9.2.3 Potential for endocrine disruption

Ferric pyrophosphate is a substance commonly considered safe and approved for use as a dietary supplement and food additive, thus negative impact on the endocrine system is not expected. In addition, ferric pyrophosphate is virtually insoluble in water and elements found in its composition - iron and phosphorus - occur naturally in the aquatic environment and living organisms and are indispensable for their proper functioning.

No studies were performed to assess the potential of Ferric pyrophosphate for endocrine disruption concerning aquatic organisms. However, although such studies were not performed using the active substance, the Fish, Early-life Stage Toxicity Test (OECD TG 210) was conducted for the foreseen plant protection product with Ferric pyrophosphate as an active substance (Code number: BW01 GB, proposed trade name VITROL GB). According to OECD (2018) Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption. OECD Publishing, Paris, this test has no endocrine-specific endpoints, however, there is limited evidence to suggest that some thyroid system disrupters are able to interfere with metamorphosis of the fish embryo to the larva in this test.

Fish, Early-life Stage Toxicity Test

Report: [redacted] 2014, The study of the long-term and chronic toxicity to fish according to OECD 210

Some more detailed information concerning the study is given in DAR Volume 3 Annex B.9: Ecotoxicology (PPP), in point B.9.3.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BW01 GB
Description: turquoise granules
Content of active substance: 3% of technical form of iron pyrophosphate (2,4% of anhydrous Ferric pyrophosphate)
2. Vehicle and/or positive control: tap water
3. Test animals:
Species: Zebrafish (*Danio rerio)*

B. STUDY DESIGN AND METHODS:

Preliminary test

Preliminary test included fish acute toxicity and the following concentrations of test item were used: 0,1; 1; 10 and 100 mg/l. Each concentration was prepared in a single replicate, and into each of them, 7 fish were introduced. The feeding of fish was suspended 24 hours before the beginning of the experiment. The experiment was carried out in a semi-static system (solutions were exchanged at 24 hours) and lasted for four days. Observations of the fish mortality and signs of intoxication were carried out on 3, 6, 24, 48, 72 and 96 hours after the start of the experiment.

Definitive test

Based on information obtained from the preliminary test, it was decided to carry out the definitive test as a limit test. One concentration of the test substance – nominal 10 mg/l was used. To obtain the suspension with the test material, granules were pulverised in in a mortar to a powder. The experiment was carried out in a semi-static system. Suspensions with the test item were exchanged by a fresh one at every 72 hours. At the beginning and at the end of the 72 hours period the concentration of iron in a solution, after filtering through a membrane filter of 0.45µm, was measured. On the basis of these measurements the concentration of the test item in the test medium was calculated.

The test was prepared in four replications with 20 eggs per each aquarium. Eggs shortly after fertilization, but

before the first cell division were used for the experiment. Fish used as a broodstock in order to obtain eggs for definitive experiment came from the same group of fish as the those used for preliminary test. The test lasted 30 days from the hatching.

The following parameters were daily observed and recorded during the experiment:

- the number of hatched larvae,
- the number of dead eggs, embryos, larvae and young fish,
- the number of larvae and fish with abnormal appearance with description of these irregularities,
- intoxication symptoms e.g.: hyperventilation, uncoordinated swimming, immobility, or unusual behaviour during feeding.

At the end of the experiment fish from each repetition of control and tested concentration were weighed and then having regard to the number of fish in the repetition, the weight of the animals were converted to one fish. Surviving fish were measured using gauge rigid with a nominal length of 1m – due to the correct shape of the tail fins of fish, the total length of the fish was reported.

II. RESULTS

Table 1. Preliminary test - the content of the test item in medium indicated from the results of ICP-OES

Nominal concentration Mg/L	The beginning of the experiment		The end of the experiment			
	0,1	100	0,1		100	
			Without aeration	With aeration	Without aeration	With aeration
Marked concentration of iron µ/L	-2,03	29,14	-3,17	-3,12	29,48	32,06
Marked concentration of test item mg/L	-0,28	4,05	-0,44	-0,43	4,09	4,45

Table 2. Preliminary test – fish mortality – raw data

	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Nominal concentration (mg/l)	Total mortality	Total mortality	Total mortality	Total mortality	Total mortality	Total mortality
control	0	0	0	0	0	0
1	0	0	0	0	0	0
10	0	0	0	0	0	0
100	0	0	0	0	0	0

Table 3. Definitive test - test item content in a solution[1]

Nominal test item content Mg/L	Iron concentration (µg/L) ICP-OES measurements				Test item content (mg/L) (calculated)			
	fresh solution		old (3-day) solution		fresh solution		old (3-day) solution	
10	mean	standard deviation	mean	standard deviation	mean	standard deviation	mean	standard deviation
	33,25	5,09	33,09	3,58	4,62	0,71	4,60	0,49

[1]The mean and standard deviation were calculated from all measurements of all four replications

Table 4. Results of the definitive test – control

Day	Number of hatched larvae				Number of dead eggs (J), embryos (E), larvae (L), fish (R)				Intoxication symptoms			
	Number of the repetition				Number of the repetition				Number of the repetition			
	1	2	3	4	1	2	3	4	1	2	3	4
1	10	9	10	11	0	0	0	0	no(1)	no	no	no
2	14	14	14	14	6 (J)	6 (J)	6 (J)	6 (J)	no	no	no	no
3	-	-	-	-	0	0	0	0	no	no	no	no
4	-	-	-	-	0	0	1 (L)	0	no	no	no	no
5	-	-	-	-	0	0	0	1 (R)	no	no	no	no
6	-	-	-	-	1 (R)	1 (R)	0	0	no	no	no	no
7	-	-	-	-	0	0	1 (R)	0	no	no	no	no
8	-	-	-	-	0	0	0	0	no	no	no	no
9	-	-	-	-	0	0	0	1 (R)	no	no	no	no
10	-	-	-	-	0	0	0	0	no	no	no	no
11	-	-	-	-	1 (R)	0	0	0	no	no	no	no
12	-	-	-	-	0	0	0	0	no	no	no	no
13	-	-	-	-	0	1 (R)	0	0	no	no	no	no
14	-	-	-	-	0	0	0	0	no	no	no	no
15	-	-	-	-	0	0	0	0	no	no	no	no
16	-	-	-	-	0	0	1 (R)	0	no	no	no	no
17	-	-	-	-	0	0	0	0	no	no	no	no
18	-	-	-	-	0	0	0	1 (R)	no	no	no	no
19	-	-	-	-	0	0	0	0	no	no	no	no
20	-	-	-	-	0	1 (R)	0	0	no	no	no	no
21	-	-	-	-	0	0	0	0	no	no	no	no
22	-	-	-	-	0	0	0	0	no	no	no	no
23	-	-	-	-	1 (R)	0	0	0	no	no	no	no
24	-	-	-	-	0	0	0	0	no	no	no	no
25	-	-	-	-	0	0	0	0	no	no	no	no
26	-	-	-	-	0	0	0	0	no	no	no	no
27	-	-	-	-	0	0	1 (R)	0	no	no	no	no
28	-	-	-	-	0	0	0	0	no	no	no	no
29	-	-	-	-	0	0	0	0	no	no	no	no
30	-	-	-	-	0	0	0	1 (R)	no	no	no	no
31	-	-	-	-	0	0	0	0	no	no	no	no
32	-	-	-	-	0	0	0	0	no	no	no	no

(1) "no" – no symptoms of intoxication or abnormal appearance of alive fish or larvae

Table 5. Results of the definitive test – test item

Day	Number of hatched larvae				Number of dead eggs (J), embryos (E), larvae (L), fish (R)				Intoxication symptoms			
	Number of the repetition				Number of the repetition				Number of the repetition			
	1	2	3	4	1	2	3	4	1	2	3	4
1	10	9	10	9	0	0	0	0	no(1)	no	no	no
2	14	13	14	14	6 (J)	7 (J)	6 (J)	6 (J)	no	no	no	no
3	-	-	-	-	0	0	0	0	no	no	no	no
4	-	-	-	-	0	0	1 (L)	0	no	no	no	no
5	-	-	-	-	1 (R)	0	0	0	no	no	no	no
6	-	-	-	-	1 (R)	0	1 (R)	1 (R)	no	no	no	no
7	-	-	-	-	0	1 (R)	0	0	no	no	no	no

8	-	-	-	-	0	0	0	0	no	no	no	no
9	-	-	-	-	0	0	0	1 (R)	no	no	no	no
10	-	-	-	-	0	0	0	0	no	no	no	no
11	-	-	-	-	0	0	1 (R)	0	no	no	no	no
12	-	-	-	-	1 (R)	0	0	0	no	no	no	no
13	-	-	-	-	0	0	0	0	no	no	no	no
14	-	-	-	-	0	1 (R)	0	0	no	no	no	no
15	-	-	-	-	0	0	0	0	no	no	no	no
16	-	-	-	-	0	0	0	0	no	no	no	no
17	-	-	-	-	0	0	0	1 (R)	no	no	no	no
18	-	-	-	-	0	1 (R)	0	0	no	no	no	no
19	-	-	-	-	0	0	1 (R)	0	no	no	no	no
20	-	-	-	-	0	0	0	0	no	no	no	no
21	-	-	-	-	0	0	0	0	no	no	no	no
22	-	-	-	-	0	0	0	1 (R)	no	no	no	no
23	-	-	-	-	1 (R)	0	0	0	no	no	no	no
24	-	-	-	-	0	0	0	0	no	no	no	no
25	-	-	-	-	0	1 (R)	0	0	no	no	no	no
26	-	-	-	-	0	0	0	0	no	no	no	no
27	-	-	-	-	0	0	0	0	no	no	no	no
28	-	-	-	-	0	0	0	0	no	no	no	no
29	-	-	-	-	0	0	0	1 (R)	no	no	no	no
30	-	-	-	-	0	0	0	0	no	no	no	no
31	-	-	-	-	0	0	0	0	no	no	no	no
32	-	-	-	-	0	0	0	0	no	no	no	no

(1) "no" – no symptoms of intoxication or abnormal appearance of alive fish or larvae

Table 6. Fish average bodyweight and body length from definitive test – control and tested concentration (10 mg/l)

Tested concentration mg/L	**Repetition 1**		**Repetition 2**		**Repetition 3**		**Repetition 4**	
	weight	**length**	**weight**	**length**	**weight**	**length**	**weight**	**length**
control	0,45	15	0,44	14	0,46	15	0,45	15
10	0,44	14	0,45	15	0,45	15	0,44	14

III. DISCUSSION AND CONCLUSIONS

The lack of any effects of the test material on the percentage hatching, the survival or growth of organisms (expressed as weight and length change) allows the conclusion that there is no indication that Ferric pyrophosphate may have the endocrine disrupting potential for aquatic organisms at tested concentration of plant protection product 4.6 mg/l (nominal – 10 mg/l). In addition, Ferric pyrophosphate is an inorganic salt, whose components – iron and phosphorus - occur naturally in the aquatic environment and living organisms and are indispensable for their proper functioning.

According to OECD (2018) Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption. OECD Publishing, Paris, the data suggests that Ferric pyrophosphate does not have thyroid activity in fish.

According to Commission Regulation (EU) 2018/605 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties, Ferric pyrophosphate, considering aquatic organisms, should be classified as not having endocrine disrupting properties.

RMS: According to the ED criteria[2], a substance shall be considered as having ED properties if it meets all of the following criteria:

2 Commission Regulation (EU) 2018/605 of 19 April 2018 setting out scientific criteria for the determination of endocrine disrupting and amending Annex II to Regulation (EC) 1107/2009. OJ L 101, 20.4.2018, p. 33–36.
Available online: http://data.europa.eu/eli/reg/2018/605/oj

a) it shows an adverse effect in non-target organisms, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub) that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
b) it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;
c) the adverse effect is a consequence of the endocrine mode of action.

In the study of the long-term and chronic toxicity to fish according to OECD TG 210 (Ziółkowska, 2014) the adverse effects were not seen in Zebrafish (Danio rerio) exposed at nominal 10 mg BW01 GB /l. Fish early life stage (FELS) toxicity test (OECD TG 210) is a method belonging to level 4 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals, revised 2017, including In vivo assays providing data on adverse effects on endocrine-relevant endpoints. The mode of action which may produce a response in this test would be alteration of thyroid function, since some thyroid system disrupters are able to interfere with metamorphosis of the fish embryo to the larva.
The lack of any effects of the test material on the percentage of hatched larvae, the survival or growth of organisms (expressed as weight and length change) shows that the first criterion, i.e. induction of adverse effect in fish was not fulfilled and results of the test indicate that ferric pyrophosphate should not considered as having endocrine disrupting properties for fish.

The EFSA experts at the Pesticide Peer Review Meeting 14 (19 September 2019) discussed the ED potential of Ferric pyrophosphate taking into account also the arguments and conclusions of the Pesticide Peer Review Meeting 11 (5 September 2019) on toxicology. Iron and phosphate ions are considered ubiquitous in the environment and are also essential for animal and plant functions. Because of the limited data package for ferric pyrophosphate, the risk assessment and hazard identification mainly relied on clinical and epidemiological studies in humans with iron salts. Available toxicity studies on ferric pyrophosphate did not identify toxicological effects. This is in line with previous evaluations of ferrous and phosphate as food additives and nutrient sources (EFSA ANS, 2010) and as pesticides active substances (EFFSA, 2012 and EFSA, 2015). For a quantitative risk assessment of ferric pyrophosphate as a plant protection product, the RMS proposed to use the safe long-term intake of iron supplements of 50 mg iron/day (for a 60 kg individual) in pregnant women as the point of departure as done previously for ferric phosphate (EFSA, 2015) and iron sulphate (EFSA, 2012).
Experts in toxicology agreed to waive ED assessment based on the scientific rational presented by RMS in the RAR.
The EFSA experts at the Pesticide Peer Review Meeting 14 (19 September 2019) in ecotoxicology noted that the substance is of poor solubility in water therefore not bioavailable to fish and amphibians. As matter of fact, solubility in water and organic solvents is as follows:

Solubility in water (state temperature, state purity and pH)	20 ± 0.5 °C (101.73%) pH 4 (24h–0.1403 mg/L; 48h–0.1648 mg/L; 72h–0.1417 mg/L) pH 7 (24h–0.0412 mg/L; 48h–0.0416 mg/L; 72h–0.039 mg/L) pH 9 (24h–0.1359 mg/L; 48h–0.1131 mg/L; 72h–0.1123 mg/L)
Solubility in organic solvents (state temperature, state purity)	20 ± 1 °C (101.73%) n-heptane < 10 [g/L] p-xylene < 10 [g/L] 1,2-dichloroethane < 10 [g/L] methanol < 10 [g/L] acetone < 10 [g/L] ethyl acetate < 10 [g/L]

There is an ELS done with the formulation (active difficult to test as it is insoluble) over 28 days and there are no effects even if the ELS is not so informative for ED.

In the ECHA/EFSA Guidance, the following is stated:
There may be cases in which due to the knowledge on the physico-chemical and (eco)toxicological properties of the substance an ED assessment does not appear scientifically necessary or testing for this purpose not technically possible (BP Regulation1, Annex IV or PPP Regulation,2 Annex, Point 1.5). In such cases, it should be justified for PPPs (Commission Regulation (EU) No 283/20137) or the general rules for adaptation of the data requirements set out in Annex IV of the BP Regulation1 shall be followed or, for PPPs, used as examples. However, it needs to be considered if possible adaptations would apply to the ED assessment in its entirety or only with respect to humans or non-target organisms.

Overall, by considering that the substance is used as food additive and nutrient and that is poorly soluble in water and therefore the test could be difficult to perform with the active, it is considered that the ED assessment can be waived. A justification is included in the RAR for waiving the generation of further data as no further testing is necessary .

B.9.2.4Acute toxicity to aquatic invertebrates

B.9.2.4.1Acute toxicity to Daphnia magna

Report: Ziółkowska A., Wickiel G. 2013; Daphnia sp. Acute Immobilization Test according to OECD Guideline No 202
Study code: 0003/0022/E
Guideline: OECD No 202
GLP: yes
Executive Summary:
The acute Daphnia sp. (Daphnia magna) immobilization test for test item, Iron pyrophosphate was conducted. 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 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 was observed, nor signs of intoxication in any replicate of tested concentration being the solubility limit concentration of the test item. Basing on the actual observations value NOEC ≥ 92 µg/l was estimated for determined concentration and NOEC ≥ 100 mg/l for nominal concentration. Reference test with reference substance - potassium dichromate was performed.

I. MATERIALS AND METHODS

A. MATERIALS:
1. Test Material: Iron pyrophosphate
Description: cream-colored powder
Batch No: 120327086
Content of active ingredient: 98 %
Storage: cool and dry place

2. Vehicle and/or positive control: ISO / potassium dichromate

3. Test animals
Species: Daphnia magna
Age: no older than 24 hours
Diet: green algae and suspension of lyophilized marine algae
Source: from a culture of research laboratory
Acclimation period: n.d.

4. Environmental conditions

Temperature: 20 ± 2 °C
Oxygen concentration: 5,99-6,51mg/l
pH: 7,24-7,63
Photoperiod: fluorescent lighting with intensity of 400 - 1000 lux, 16 h day and 8 h night

B. STUDY DESIGN AND METHODS:

1. In life dates: 17-19.06.2013

2. Experimental treatments

Preliminary test

In the preliminary test the following concentrations of test item were used: 11,5; 23; 46 and 92 µg/l. Each concentration was prepared in two replicates, 10 organisms introduced into each replicate, not being first brood progeny of the motherhood group. The feeding was suspended 24 hours before the beginning of the experiment. In the experiment, the animals no older than 24 hours were used. 100 ml glass beakers were used as test vessels, the volume of test solution was 100 ml in each beaker. Beakers were covered with material in order to prevent the possible contamination. The test was carried out in a static system and lasted for 48 hours.

Due to the poor solubility of test item in medium, a stock solution was prepared by stirring 100 mg of test item in the 1 liter of medium for 72 hours using a mechanical stirrer. Afterwards, the solution was filtered through a membrane filter with a pore size of 0,45 µm. Further concentrations of the test material were obtained by diluting twice the previous concentration.

Definitive test

Due to the lack of the immobilization of the organisms in a preliminary test, a limit test was applied using one concentration – 92µg/l which is the solubility limit of the test item in the medium. Stock solution was prepared by adding 100 mg of the test item into 1 liter of ISO medium and stirring it for 72 hours in a experiment's temperature using a mechanical stirrer. After stirring the solution was filtered through a membrane filter with a pore size of 0,45 µm.

The test was conducted in the 100 ml glass beakers. Beakers were covered with material in order to prevent the possible contamination, and they were arranged randomly in accordance with generated number sequence. The test lasted for 48 hours. The test was prepared in four replicates with 5 organisms introduced into each replicate. In the experiment, the animals no older than 24 hours were used, not being the first brood progeny of the motherhood group. The feeding of organisms was suspended 24 hours before the beginning of the test. Based on results from stability test, the definitive experiment was conducted in static exposure system, the solution was not replaced with a fresh one.

3. Observations

During the experiment, the water temperature was recorded (additional vessel was positioned directly next to the test vessels). At the beginning and at the end of experiment, as well as after every 24 hours, pH and dissolved oxygen values were measured. Observations of Daphnia immobilization, appearance and behaviour changes in each vessel after 24h and 48h after the beginning of the experiment were performed. Observations were carried out every day within ± 1 hour after the start of the experiment. No immobilization and no signs of intoxication were observed during the study.

4. Statistical calculations

ECx, NOEC and LOEC values were calculated by ToxRat Professional.

5. Deviations from the Guideline

During the experiment the medium temperature decreased for 1 hour below the lower limit (22°C) by 0,2°C and for five hours by 0,1°C. As the decrease was slight and lasted for a short time in relation to the total time of the experiment, it was concluded that the deviation did not affect the test and final results.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.2-4 Preliminary test - number of immobilized Daphnia - raw data

Concentration of test item [µg/l]	Replication	24 hours		48 hours	
		Number of immobilized	Total % of immobilized	Number of immobilized	Total % of immobilized
Control	1	0	0	0	0

	2	0		0	
11,5	1	0	0	0	0
	2	0		0	
23	1	0	0	0	0
	2	0		0	
46	1	0	0	0	0
	2	0		0	
92	1	0	0	0	0
	2	0		0	

Table 9.2-5 Definitive test - number of immobilized Daphnia - raw data

Concentration of test item [µg/l]	Replication	24 hours		48 hours	
		Number of immobilized	Total % of immobilized	Number of immobilized	Total % of immobilized
Control	1	0	0	0	0
	2	0		0	
	3	0		0	
	4	0		0	
92	1	0	0	0	0
	2	0		0	
	3	0		0	
	4	0		0	

B. OBSERVATIONS

Due to this the definitive test was performed as the limit test using single concentration of the test substance – 92µg/L which is the limit of its solubility. In none of the tested item concentrations the immobilization of Daphnia was observed, neither signs of intoxication.

III. CONCLUSIONS

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 was observed, nor signs of intoxication in any replicate of tested concentration being the solubility limit concentration of the test item. Basing on the actual observations value NOEC ≥ 92 µg/l was estimated for determined concentration and NOEC ≥ 100 mg/l for nominal concentration.

RMS comments:

The study performed in GLP conditions and according OECD TG 202 is considered valid and established endpoints may be used for risk assessment, however it should be noted that at the highest tested concentration of 92 µg/l, being it's solubility limit, and corresponding to nominal concentration of 100 mg/l, no toxic effect were observed. No significant deviations from the test guideline were noted. All validity criteria were met. The definitive test was performed with saturated solution of the test item equal to 92 µg/L taken from nominal stock solution containing 100 mg of iron pyrophosphate in 1 L litre of ISO medium.

The study is considered acceptable with following endpoints:

48h EC_{50} >92 µg/l being a solubility limit of iron pyrophosphate in the stock solution containing 100 mg of iron pyrophosphate in 1 L litre of ISO medium.
Since no signs of immobilisation of Daphnia, neither other signs of intoxication were observed during 48 h test a NOEC (no observed effect concentration) of iron pyrophosphate in these test conditions amounts to ≥ 92 µg/l being a solubility limit of iron pyrophosphate in the solution containing nominally 100 mg of iron pyrophosphate in 1 L litre of ISO medium.

B.9.2.4.2 Acute toxicity to an additional aquatic invertebrate species

In accordance with point 8.2.4. of Commission Regulation (EU) No. 283/2013 setting out the data requirements for active substances, acute toxicity study for an additional species of aquatic invertebrates is not required.

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

B.9.2.5.1 Reproductive and development toxicity to *Daphnia magna*

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, based on the EU Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev.4) a study of reproductive and developmental toxicity to *Daphnia magna* was conducted for the BW01 GB formulation.

B.9.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

In accordance with point 8.2.5. of Commission Regulation (EU) No. 283/2013 setting out the data requirements for active substances, reproductive and developmental toxicity study for an additional species of aquatic invertebrates is not required.

B.9.2.5.3 Development and emergence in *Chironomusriparius*

In accordance with point 8.2.5. of Commission Regulation (EU) No. 283/2013 setting out the data requirements for active substances, additional study of long-term and chronic toxicity on relevant species of animals that are not molluscs such as *Chironomus spp*. is not required.

B.9.2.5.4 Sediment dwelling organisms

Based on the information presented in point B.8.2, a study of the effect on sediment-dwelling organisms is not required.

B.9.2.6Effects on algal growth

Report: Heisterkamp I. 2015, Alga Growth Inhibition Test according to OECD 201 (23 March 2006)
Study code: 1040
Guideline: OECD 201
GLP: yes

Executive Summary:

A growth inhibition test with *Pseudokirchneriellasubcapitata* was conducted according to OECD 201 (23 March 2006) 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 and 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.

Exposure of *Pseudokirchneriellasubcapitata* 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 $ErLR_{50}$ and $EyLR_{50}$ 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.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Ferric pyrophosphate
Description: powder
Batch No: 120327086
Content of active ingredient: 101.73%

2. Vehicle and/or positive control: AAP / potassium dichromate

3**. Test animals**
Species: green alga *Pseudokirchneriellasubcapitata* (Strain No. 61.81 SAG)
Age: n.a.
Diet: algal growth medium (according to OECD 201)
Source: Culture Collection of Algae at the University of Goettingen
Acclimation period: The strain used for this study has been cultured in the laboratory of Hydrotox GmbH since November 2014

4. Environmental conditions
Temperature: 23,5-23,8°C

Oxygen concentration: no data
pH: 8,1
Photoperiod: 99.5 µE/m² s +/- 4.7%

B. STUDY DESIGN AND METHODS:

1. In life dates: 06.08 – 25.09.2015

2. Experimental treatments

Due to the low water solubility a saturated solution of the test item was prepared in AAP medium by stirring for 24 h. The dilution of the test solution by the algal inoculum has to be taken into account. Therefore, the loading rate was prepared factor 1.11 higher than intended in the test. By addition of the algal inoculum suspension the loading rate intended was achieved. A 111 mg/L loading rate was prepared by weighing 166.7 mg of the test item in 1500 mL AAP-medium and stirred for a period of 24 hours with an overhead high-grade stainless steel radial impeller (about 500 rpm) obtaining a depth of the vortex of about 1/3 of the total height of the liquid. After stirring for 24 h, the loading rate was filtrated with a 0.45 µm membrane filter (cellulose acetate) to remove non-dissolved particles and was used to make a serial dilution. Further test concentrations were prepared by diluting the filtered stock solution with AAP-medium.

The test solutions (45 mL) were transferred into the test vessels (100 mL wide-necked Erlenmeyer flask). To each test vessel 5 mL of the algae inoculum suspension (0.7 x 105 algae mL^{-1}) were added to obtain an algae starting concentration of 7 x 103 algae mL^{-1}. For the controls, 5 mL algae inoculum suspension was added to 45 mL algal medium. For the chemical analysis of the test item, additional test vessels without algae have been prepared. Algal medium was added to these additional vessels instead of inoculum suspension to avoid an influence of the organisms on the analytical result.

3. Observations

The algal growth was determined by analysing the chlorophyll fluorescence as a measure for algal biomass. After 0, 24, 48, and 72 hours, 200 µl samples were taken out of each test vessel and chlorophyll fluorescence was measured in a 96-well micro-plate for each sample using a fluorescence micro-plate reader (excitation wave length: 465 nm, emission wave length: 670 nm). The measured chlorophyll fluorescence is converted into cell counts per mL by means of a correlation factor. This correlation factor is determined twice a year during quality check by measuring different cell densities with micro-plate fluorescence reader and coulter counter and subsequent calculation of a calibration curve. The algal count was determined for a cell size between 3 and 9 µm using a Coulter Counter Z2.

According to results of the range-finder and in accordance with the sponsor, five nominal loading rates between 6.25 mg/L and 100 mg/L with a spacing factor of 2 were chosen for the GLP study. The test vessels were prepared in three replicates and the control vessels were prepared in six replicates. The specific growth rate and yield and their percent inhibition compared to the controls were calculated for each replicate after 72 hours.

4. Statistical calculations

- Shapiro-Wilk's test on normal distribution
- Levence's test on variance homogeneity
- Williams Multiple Sequential t-test procedure
- probit analysis

5. Deviations from the Guideline

None

II. RESULTS AND DISCUSSION

The algae test was performed with five nominal loading rates between 6.25 mg/L and 100 mg/L.
The following results were obtained:

Growth Rate:		Yield:	
$E_rLR_{50(72h,nominal)}$:	> 100 mg/L	$E_yLR_{50(72h,nominal)}$:	> 100 mg/L
$E_rLR_{20(72h,nominal)}$:	> 100 mg/L	$E_yLR_{20(72h,nominal)}$:	> 100 mg/L
$E_rLR_{10(72h,nominal)}$:	> 100 mg/L	$E_yLR_{10(72h,nominal)}$:	> 100 mg/L
$LOE_rLR_{(72h,nominal)}$:	> 100 mg/L	$LOE_yLR_{(72h,nominal)}$:	> 100 mg/L
$NOE_rLR_{(72h,nominal)}$:	≥ 100 mg/L	$NOE_yLR_{(72h,nominal)}$:	≥ 100 mg/L

The accompanying analysis was based on the detection of iron. The geometric mean of measured iron concentrations ranged from 0.0280 mg/L in the control to 0.0351 mg/L in the highest loading rate. Results expressed as geometric mean of the ferric pyrophosphate concentration are as following:

Growth Rate:

> 0.0212 mg/L

$E_rLR_{50(72h,geometric\ meanFe4(P2O7)3)}$: > 0.0212 mg/L
$E_rLR_{20(72h,\ geometric\ mean\ Fe4(P2O7)3)}$: > 0.0212 mg/L
$E_rLR_{10(72h,\ geometric\ mean\ Fe4(P2O7)3)}$: > 0.0212 mg/L
$LOE_rLR_{(72h,geometric\ meanFe4(P2O7)3)}$: ≥ 0.0212 mg/L
$NOE_rLR_{(72h,geometricmean\ Fe4(P2O7)3)}$:

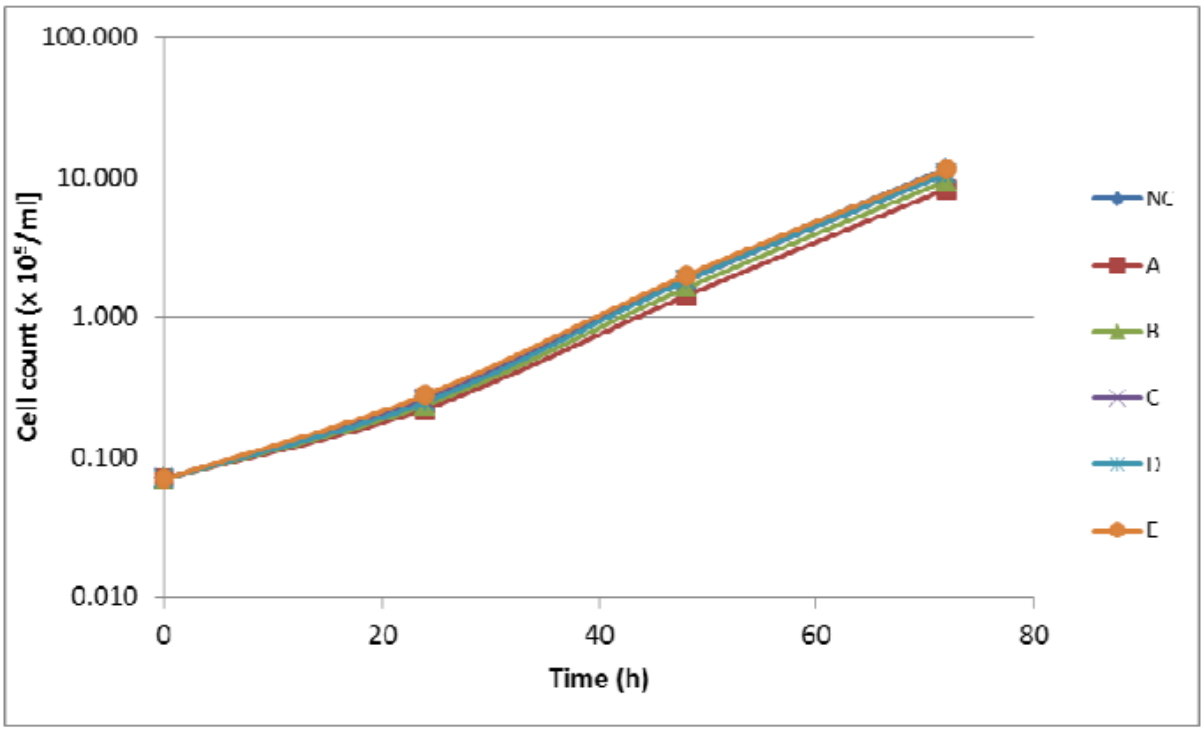
Yield:
$E_yLR_{50(72h,geometric\ meanFe4(P2O7)3)}$: > 0.0212 mg/L
$E_yLR_{20(72h,\ geometric\ mean\ Fe4(P2O7)3)}$: > 0.0212 mg/L
$E_yLR_{10(72h,\ geometric\ mean\ Fe4(P2O7)3)}$: > 0.0212 mg/L
$LOE_yLR_{(72h,geometric\ meanFe4(P2O7)3)}$: > 0.0212 mg/L
$NOE_yLR_{(72h,geometricmean\ Fe4(P2O7)3)}$: ≥ 0.0212 mg/L

Table 9.2-6 presents the mean growth rates and mean yields and the inhibitions of the five nominal loading rates compared to the control after 72 h.

Table 9.2-6 Mean growth rates and mean yields and their inhibitions compared to the control after 72 h of exposure

	Control	A	B	C	D	E
Nominal loading rate (mg/L)	0	6.25	12.5	25	50	100
Number of replicates	6	3	3	3	3	3
Mean growth rate	1.70	1.59	1.64	1.67	1.67	1.70
Mean yield	11.53	8.21	9.41	10.55	10.56	11.29
Mean inhibition of growth rate (%)	-	6.6	3.9	1.7	1.7	0.4
Mean inhibition of yield (%)	-	28.8	18.4	8.5	8.4	2.1

Figure 9.2-1 Growth curves for the negative control (NC) and the five tested nominal loading rates 6.25 mg/L (A), 12.5 mg/L (B), 25 mg/L (C), 50 mg/L (D) and 100 mg/L (E). Cell counts at 0, 24, 48 and 72 h were determined from the measured fluorescence data.



Growth curves show that the algal growth in the loading rates was similar to the control resulting in only minor inhibition rates of 0.4 to 6.6 %. The morphology of the algal cells in the five loading rates as well as in the control showed no abnormality.

The pH-values measured at the beginning and at the end of the test (after 72 h) are reported in Table 9.2-7.

Table 9.2-7 Measured pH-values at the beginning (0 h) and at the end of the test (72 h)

	Control	A	B	C	D	E
Nominal Loading rate	0	6.25	12.5	25	50	100
0 h	7.0	7.1	7.1	7.1	7.1	7.1
72 h	7.4	7.5	7.5	7.5	7.5	7.5

Chemical analysis was based on the detection of iron. Measured iron concentrations in the loading rates were very similar to the iron content in the control. At 0 h, iron concentration in the control was 0.0336 mg/L, which is very close to the intended iron content in the 1-x AAP medium of 0.0331 mg/L. The 100 mg/L loading rate contained 0.0382 mg/L at 0 h, thus only 0.0046 mg/L more iron than the AAP medium (Table 9.2-8). This indicates that almost no additional iron dissolved in the medium. Measured iron concentrations in the dilutions (from 50 mg/L to 6.25 mg/L loading rates) decreased with increasing dilution. Iron concentration after 72 h were lower than the initial values resulting in recovery rates from 84.3% in the highest loading rate to 69.6 % in the control. As recovery rates were mostly < 80% the geometric mean of the iron concentrations from 0 h to 72 h were calculated. Measured iron concentrations in the test vessels with algae showed slightly lower values than in test vessels without algae. This may either be due to the uptake of iron by the algae or due to loss of iron by filtration of the samples to remove the algal cells prior chemical analysis. The control standard of 0.1 mg/L was analysed with 0.0861 mg/L and is thus within the accepted variation range.

Table 9.2-8 Results of the accompanying iron analysis

Nominal loading rate (mg/L)	Control	6.25	12.5	25	50	100
Time (h)	Measured iron concentration in mg/L					
0 h	0.0336	0.0335	0.0342	0.0346	0.0366	0.0382
72 h	0.0234	0.0256	0.0263	0.0259	0.0277	0.0322
72 h*	0.0193	0.0244	0.0242	0.0235	0.0227	0.0213

Recovery rates 0-72 h (%)	69.6	76.4	76.9	74.9	75.7	84.3
Geometric mean 0-72 h (mg/L)	0.0280	0.0293	0.0300	0.0299	0.0318	0.0351

*from test vessels with algae, samples were filtered with a 0.45 µm membrane filter
Limit of quantification: 0.0074 mg/L

The results of the iron analysis were control-corrected and the geometric mean of the corrected value was calculated and converted according to the stechiometry of ferric pyrophosphate (Table 9.2-9).

Table 9.2-9 Iron content control-corrected and converted by the stechiometry of ferric pyrophosphate

Nominal loading rate (mg/L)	Control	6.25	12.5	25	50	100
Time (h)	Control-corrected iron concentration in mg/L					
0 h	0	-0.0001	0.0006	0.0010	0.0030	0.0046
72 h	0	0.0022	0.0029	0.0025	0.0043	0.0088
Geometric mean iron content control-corrected 0-72 h (mg/L)		n.d.	0.0013	0.0016	0.0036	0.0064
Geometric mean ferric pyrophosphate (Fe4(P2O7)3) (mg/L)		n.d.	0.0044	0.0053	0.0120	0.0212

III. CONCLUSIONS

Exposure of *Pseudokirchneriellasubcapitata* 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.

RMS comments:
The study of alga growth inhibition performed on *Pseudokirchneriellasubcapitata* in GLP conditions and according OECD TG 201 (March 2006) is considered valid and established endpoints may be used for risk assessment, however it should be noted that at the highest tested concentration of 0,0212 mg/L being a solubility limit of iron pyrophosphate in this test, corresponding to nominal concentration of 100 mg/l, no toxic effects were observed..

No significant deviations from the test guideline were noted. All validity criteria were met. The test was performed with a range of several concentrations, including a saturated solution of the test item equal to 0.0212 mg/L equivalent to nominal stock solution containing 100 mg of iron pyrophosphate in 1 L.

The study is considered acceptable with following endpoints:

E_rLR_{50} and E_yLR_{50} were above > 0.0212 mg/L (corresponding to 100 mg a.s./L, nominal)
NOELR was above > 0.0212 mg/L (corresponding to 100 mg a.s./L, nominal)

0.0212 mg/L was measured a solubility limit of iron pyrophosphate in the nominal solution containing 100 mg of iron pyrophosphate in 1 L of AAP medium.

B.9.2.6.2Effects on growth of an additional algal species

In accordance with point 8.2.6. of Commission Regulation (EU) No. 283/2013 setting out the data requirements for active substances, study of the effect on the growth of additional algae species is not required.

B.9.2.7Effect on aquatic macrophytes

In accordance with point 8.2.7. of Commission Regulation (EU) No. 283/2013 setting out the data requirements for active substances, study of the effect on aquatic macrophytes is not required.

B.9.2.8 Further testing on aquatic organisms

Based on the obtained study results and lack of toxic properties of ferric pyrophosphate and BW01 GB towards aquatic organisms, further studies on aquatic organisms are considered unnecessary.

B.9.3 Effects on Arthropods

B.9.3.1 Effects on bees

B.9.3.1.1 Acute toxicity

B.9.3.1.1.1 Acute oral toxicity

Report: A. Ziółkowska, 2013,Honeybees, Acute Oral Toxicity Test according to OECD 213
Study code: 0003/0026/E
Guideline: OECD 213
GLP: yes
Executive Summary:
The acute oral toxicity study of the test item, Iron pyrophosphate for honeybee (*Apismelliferacarnica*) was conducted. The aim of the study was to determine the dose that would cause mortality of 50% of the population (LD_{50}) after 24 and 48 hours.
The test material is non-toxic in the dose 100 µg/bee being the maximal dose recommended by the OECD Guideline No 213. In the definitive study, mortality among the population was insignificant and in any of the tested doses did not exceed 50%. There were no signs of intoxication in any of the replicates of the tested doses. Physiological condition of the bees was confirmed in the study with the reference substance – dimethoate.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Iron pyrophosphate
Description: cream-colored powder
Batch No: 120327086
Storage: cool and dry place

2. Vehicle and/or positive control: 0,5% methylcellulose solution / dimethoate

3. Test animals
Species: honeybee, *Apismelliferacarnica*
Age: approx. 3-5 weeks after emerging the pupa
Source: from agriturism farm "U Godka", Spławie, wielkopolska province
Acclimation period: acclimation was not carried out

4. Environmental conditions
Temperature: 25,4 – 25,6°C
Humidity: 61 – 67 %
Photoperiod: study was conducted in darkness

B. STUDY DESIGN AND METHODS:
1. In life dates: 5/08 – 15/08/13
2. Experimental treatments

Preliminary test
The experiment lasted for 48 hours and consisted of a single oral administration to the test group 400µl of 50% sucrose solution containing a dose of the test material in an appropriate concentration, suspended in a 0,5% methylcellulose solution. Doses were administered through a glass capillary. The preliminary test was carried out in control and five concentrations of the test material: 2,5g/l, 0,25g/l, 0,025g/l and 0,0025g/l corresponding to the following doses: 100 µg/bee; 10 µg/bee, 1 µg/bee and 0,1 µg/bee. Into each 20x20x20 cm big cage, 10 bees were introduced. Each dose and control consisted of three replicates.

Definitive test
The experiment consisted of a single oral administration to the test group 400µl of 50% sucrose solution containing a dose of the test material in an appropriate concentration, suspended in a 0,5% methylcellulose solution and then observing mortality within 48 hours. The definitive test was carried out in control and the following concentrations of the test material: 2,5g/l, 1,25g/l, 0,625g/l, 0,312g/l, 0,156g/l and 0,078g/l corresponding to the following doses: 100µg/bee; 50µg/bee, 25µg/bee, 12,5µg/bee, 6,25µg/bee and 3,125µg/bee.
Reference test

Experience with reference substance – dimethoate was performed. The following concentrations of reference substance were used: 0,04g/l, 0,02g/l, 0,01g/l and 0,005g/l and 0,0025g/l corresponding to the following doses: 0,4 µg/bee, 0,2 µg/bee, 0,10 µg/bee, 0,05 µg/bee and 0,025 µg/bee.

3. Observations

Observations of bees mortality, as well as behaviour changes in each cage were made after 4, 24 and 48 hours after the beginning of the experiment. Each day observations were made within ±1h from the time that the experiment started.

4. Data analysis

On the basis of the obtained data the calculations were made according to OECD Guideline No 213 using a statistical program - ToxRat Professional using probit analysis.

5. Deviations from the Guideline

Deviations – none.

II. RESULTS AND DISCUSSION

III.

A. FINDINGS

Table 9.3-1 Preliminary test – number of dead bees – raw data

Dose of test item [µg/bee]	Replicate	Number of introduced bees	4 hours		24 hours		48 hours	
			Number of dead bees	Signs of intoxication	Number of dead bees	Signs of intoxication	Number of dead bees	Signs of intoxication
Control	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
0,1	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
1	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
10	1	10	0	no symptoms	0	no symptoms	1	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
100	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	1	no symptoms

Table 9.3-2 Definitive test – number of dead bees – raw data

Dose of test item [µg/bee]	Replicate	Number of introduced bees	4 hours		24 hours		48 hours	
			Number of dead bees	Signs of intoxication	Number of dead bees	Signs of intoxication	Number of dead bees	Signs of intoxication
Control	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
3,125	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
6,25	1	10	0	no symptoms	0	no symptoms	2	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	1	no symptoms
12,5	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
25	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms
50	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	1	no symptoms
100	1	10	0	no symptoms	0	no symptoms	0	no symptoms
	2	10	0	no symptoms	0	no symptoms	0	no symptoms
	3	10	0	no symptoms	0	no symptoms	0	no symptoms

Table 9.3-3LDx values for bees mortality* – definitive test

	Exposure time 24 h	Exposure time 48 h
LD_{10} [µg/bee]	n.d.**	n.d.**

LD_{20} [µg/bee]	n.d.**	n.d.**
LD_{50} [µg/bee]	n.d.**	n.d.**
NOEC [µg/bee]	≥ 100	≥ 100
LOEC [µg/bee]	> 100	> 100

* values calculated by ToxRat Professional
**n.d. – not determined due to mathematical reasons or inappropriate data

B. OBSERVATIONS
During the experiment no signs of intoxication were observed (e.g. unusual behavior, rejection of the sugar solution with the test material, or reduce of the intake of a 50% solution of sugar in methylcellulose compared to the control group).

III. CONCLUSIONS
The test material is non-toxic in the dose 100 µg/bee being the maximal dose recommended by the OECD Guideline No 213. In the definitive study, mortality among the population was insignificant and in any of the tested doses did not exceed 50%. There were no signs of intoxication in any of the replicates of the tested doses.

RMS comments:
The study of acute oral toxicity of iron pyrophosphate on honeybees was performed in GLP conditions and according OECD TG 213. No significant deviations from the test guideline were noted. All validity criteria were met. The study is considered valid and established endpoint may be used for risk assessment. No mortality of bees or toxic symptoms were observed within 48h after dosing of test item in doses 3.125 – 100 µg/bee. The study is considered acceptable with following endpoint:

48h oral LD_{50} for bees > 100 µg a.s. /bee

B.9.3.1.1.2 Acute contact toxicity

Report: J. Winkler 2013, Honeybees, Acute Contact Toxicity Test according to OECD 214
Study code: 0003/0025/E
Guideline: OECD 214
GLP: yes
Executive Summary:
The acute contact toxicity study of the test item, Iron pyrophosphate for honeybee (*Apismellifera*, L.) was conducted. The aim of the study was to determine the dose that would cause mortality of 50% of the population (LD50) after 24 and 48 hours. The test material is non-toxic in the dose 100 µg/bee being the maximal dose recommended by the OECD Guideline No 214. In the main study, mortality among the population was insignificant and in any of the tested doses did not exceed 50%. There were no signs of intoxication in any of the replicate of the tested doses.

I. MATERIALS AND METHODS

A. MATERIALS:
1. Test Material: Iron pyrophosphate
Description: cream-colored powder
Batch No: 120327086
Storage conditions: cool and dry place

2. Vehicle and/or positive control: 0,5% methylcellulose solution / dimethoate

3. Test animals
Species: honeybee, *Apismelliferacarnica*
Age: 3- 5 weeks
Source: agritourism “U Godka”, Spławie, wielkopolska province
Acclimation period: not carried out

4. Environmental conditions

Temperature: 25°C±2°C
Humidity: 50-70%
Photoperiod: 24 h dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 15/08 – 22/08/2013

2. Experimental treatments

The test material was prepared as a stock suspension in 0,5% methylcellulose solution

Preliminary test

The following range of doses was used: 100 µg/bee, 10 µg/bee, 1 µg/bee and 0,1 µg/bee and 0,5% methylcellulose solution was used as control. Each dose as well as control were prepared in three replicates. As test vessels, a well-ventilated, 20x20x20 cm cages were used. 10 bees were introduced into each cage. The experiment lasted for 48h.;

Main test

During the experiment a single dose in volume of 1µl of tested item suspended in 0,5% methylcellulose solution was applied on the thorax of each bee, thereafter the observations were made within 48 hours. Before the application of the test item, bees were anesthetized with carbon dioxide. The test was carried out in five doses with a factor 2,0: 6,25 µg/bee; 12,5 µg/bee; 25 µg/bee; 50 µg/bee; 100 µg/bee and control being the 0,5% methylcellulose solution. Each dose and control consisted of three replicates. Into each 20x20x20 cm cage, 10 bees were introduced. The study lasted for 48 hours.

Reference test

The test with reference substance – dimethoate was performed. The following doses of reference substance with a factor 2,0 were used: 0,025 µg/bee, 0,05 µg/bee, 0,10 µg/bee, 0,20 µg/bee and 0,40 µg/bee. Also, deionized water was used as a control. Doses were prepared by suspending the stock amount of test material in a deionized water, and therefore preparing the 2-fold dilutions. The study was performed in three replicates for each dose as well as control, with 10 bees introduced into each replicate.

3. Observations

Observations of bees mortality, as well as behavior changes in each cage were made after 4, 24 and 48 hours after the beginning of the experiment. Each day observations were made within ±1h from the time that the experiment started.

4. Data analysis

On the basis of the obtained data the calculations were made according to OECD Guideline No 214.

LD_{50}, LD_{20} and LD_{10} values for bees mortality along with NOEC and LOEC values were calculated using a statistical program - ToxRat Professional. ToxRat Professional used the following statistical analyses for the calculations:

- probit analysis using linear max. likelihood regression
- Fisher's exact binomial test with Bonferroni correction.

5. Deviations to the Guideline/Research Plan

Not applicable.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.3-4 Preliminary test – number of dead bees – raw data

Dose of test item [µg/bee]	Replicate	4 hours		24 hours		48 hours	
		Number of dead bees	Total % of dead bees	Number of dead bees	Total % of dead bees	Number of dead bees	Total % of dead bees
Control	1	0	0	0	0	0	0
	2	0		0		0	
	3	0		0		0	
0,1	1	0	0	0	0	0	0
	2	0		0		0	
	3	0		0		0	
1	1	0	0	0	0	0	0
	2	0		0		0	
	3	0		0		0	

10	1	0	0	0	0	0	3,33
	2	0		0		1	
	3	0		0		0	
100	1	0	0	0	0	0	3,33
	2	0		0		0	
	3	0		0		1	

Table 9.3-5 Main test – number of dead bees – raw data

Dose of test item [µg/bee]	Replicate	Number of introduced bees	4 hours		24 hours		48 hours	
			Number of dead bees	Total % of dead bees	Number of dead bees	Total % of dead bees	Number of dead bees	Total % of dead bees
Control	**1**	**10**	0	0	0	0	0	0
	2	**10**	0		0		0	
	3	**10**	0		0		0	
6,25	**1**	**10**	0	0	0	0	0	0
	2	**10**	0		0		0	
	3	**10**	0		0		0	
12,5	**1**	**10**	0	0	0	0	1	3,33
	2	**10**	0		0		0	
	3	**10**	0		0		0	
25	**1**	**10**	0	0	0	0	0	3,33
	2	**10**	0		0		0	
	3	**10**	0		0		1	
50	**1**	**10**	0	0	0	0	0	3,33
	2	**10**	0		0		0	
	3	**10**	0		0		1	
100	**1**	**10**	0	0	0	0	0	3,33
	2	**10**	0		0		1	
	3	**10**	0		0		0	

Table 9.3-6LDx values for bees mortality – main test.*

	Exposure time 24 h	Exposure time 48 h
LD_{10} [µg/bee]	n.d.**	n.d.**
LD_{20} [µg/bee]	n.d.**	n.d.**
LD_{50} [µg/bee]	n.d.**	n.d.**
NOEC [µg/bee]	≥100	≥100
LOEC [µg/bee]	>100	>100

* values calculated by ToxRat Professional
**n.d. – not determined due to mathematical reasons or inappropriate data

Table 9.3-7 Reference test – number of dead bees – raw data

Dose of test item [µg/bee]	Replicate	4 hours		24 hours		48 hours	
		Number of dead bees	Total % of dead bees	Number of dead bees	Total % of dead bees	Number of dead bees	Total % of dead bees

Control	1	0	0	0	0	0	0
	2	0		0		0	
	3	0		0		0	
0,025	1	0	0	0	0	0	0
	2	0		0		0	
	3	0		0		0	
0,05	1	0	0	0	0	1	6,67
	2	0		0		0	
	3	0		0		1	
0,1	1	0	0	1	20	1	23,3
	2	0		3		3	
	3	0		2		3	
0,2	1	2	13,3	8	83,3	8	90
	2	1		8		9	
	3	1		9		10	
0,4	1	2	20	10	100	10	100
	2	3		10		10	
	3	1		10		10	

Table 9.3-8LDx values for bees mortality – reference test.*

	Exposure time 24 h	Exposure time 48h
LD_{10} [µg/bee]	0,087 (0,065-0,102)**	0,067 (0,050-0,081)**
LD_{20} [µg/bee]	0,102 (0,082-0,117)**	0,082 (0,065-0,097)**
LD_{50} [µg/bee]	0,138 (0,120-0,159)**	0,121 (0,103-0,141)**
NOEC [µg/bee]	0,050	0,050
LOEC [µg/bee]	0,1	0,1

* values calculated by ToxRat Professional
**- 95% confidence interval

B. OBSERVATIONS
During the experiment no signs of intoxication were observed.

III. CONCLUSIONS

The test material is non-toxic in the dose 100 µg/bee being the maximal dose recommended by the OECD Guideline No 214. In the main study, mortality among the population was insignificant and in any of the tested doses did not exceed 50%. There were no signs of intoxication in any of the replicate of the tested doses.

RMS comments:
The study of acute contact toxicity for honeybees was performed in GLP conditions and according OECD TG 214. No significant deviations from the test guideline were noted. All validity criteria were met. The study is considered valid and established endpoints may be used for risk assessment. A low percentage of mortality of bees was observed (3.33%; 1/30 bees) within 48h after dosing of test item in doses 12.25 – 100 µg/bee without dose-response relationship, thus not related to toxicity of test item. The study is considered acceptable with following endpoint:

48h contact LD_{50} for bees > 100 µg a.s. /bee

B.9.3.1.2 Chronic toxicity to bees
Since the acute toxicity study in bees showed that the material investigated is non-toxic, and the PPP studied has a form of non-dusty granules applied directly to soil, the use of the PPP containing ferric pyrophosphate does not pose a risk to bees. Therefore, a study of chronic toxicity to bees was not conducted.

B.9.3.1.3 Effects on honeybee development and other honeybee life stages
Since the acute toxicity study in bees showed that the material investigated is non-toxic, and the PPP studied has a form of non-dusty granules applied directly to soil, the use of the PPP containing ferric pyrophosphate does not pose a risk to bees. Therefore, a study of the effect on the development and other life stages of honey bees was not conducted.

B.9.3.1.4 Sub-lethal effects
Since the acute toxicity study in bees showed that the material investigated is non-toxic, and the PPP studied has a form of non-dusty granules applied directly to soil, the use of the PPP containing ferric pyrophosphate does not pose a risk to bees. Therefore, a study of sub-lethal effects in bees was not conducted.

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

B.9.3.2.1. Effects on Aphidiusrhopalosiphi

A study of the effect on *Aphidiusrhopalosiphi* was not conducted because this is a leaf-dwelling species, whereas the PPP investigated is applied directly to soil, thus the likelihood of exposure and risk are extremely low. Instead, a study of the effect of BW01 GB on *Poeciluscupreus* (B.9.5.2.1) and *Aleocharabilineata* (B.9.5.2.2) was conducted.

B.9.3.2.2. Effects on Typhlodromuspyri
A study of the effect on *Typhlodromuspyri*was not conducted because this is a leaf-dwelling species, whereas the PPP investigated is applied directly to soil, thus the likelihood of exposure and risk are extremely low.

Instead, a study of the effect of BW01 GB on *Poeciluscupreus* (B.9.5.2.1) and *Aleocharabilineata* (B.9.5.2.2) was conducted.

In addition studies of toxicity of ferric pyrophosphate to two soil dwelling arthropod species were conducted (see: B.9.4.2)

B.9.4 Effects on Non-target Soil Meso- and Macrofauna

B.9.4.1 Earthworms– sub-lethal effects

Report: Winkler J. 2014, Earthworm reproduction test according to OECD 222
Study code: 0003/0028/E
Guideline: OECD TG 222
GLP:yes
Executive Summary:
The reproduction test (study of the sub-lethal effects) of the test item, Iron pyrophosphate for earthworm (*Eiseniafoetidafoetida*) was conducted. Obtained study results and performed statistical calculations indicate, that test item - iron pyrophosphate, tested in it's maximal - according to OECD 222 – applicable concentration 1000mg/kg, has no effect on survival and growth (expressed as the change in body weight) of adult earthworms during the 4 weeks of experiment as well as reproduction expressed as young earthworms number at the end of the study. Test item shows no toxicity against earthworms *Eiseniafoetidafoetida*. Basing on the obtained results, the NOEC value (NOEC ≥ 1000mg/kg) for reproduction was calculated.

I. MATERIALS AND METHODS

A. MATERIALS:
1. Test Material:Iron pyrophosphate
Description: cream-colored powder
Batch No: 120327086
Purity: min. 98%
Storage: cool and dry place

2. Vehicle and/or positive control: artificial soil / carbendazim

3. Test animals

Species: earthworm (*Eiseniafoetidafoetida*)
Age: 6 – 7 months
Weight: 322 - 462 mg
Source: HodowlaDżdżownicKalifornijskich, LucynaiSławomirSiudziński, KępaOborska 20, 05-520 KonstancinJeziorny
Acclimation period: 24 h
Diet: -

4. Environmental conditions

Temperature: 17,90ºC - 21,20ºC
Humidity: artificial soil humidity at the beginning of the test 35,56%-36,10%, at the end of the test 34,02%-33,12%;
Photoperiod: 400-800 lux at daily light cycle 16h light: 8h dark

B. STUDY DESIGN AND METHODS:

1. In life dates: 12.12.13 – 05.02.14

2. Experimental treatments

1.Definitive test

The definitive test principle was an application of test item concentration into the artificial soil, thereby obtaining the appropriate concentration of test item in relation to dry weight of the artificial soil, transferring it into test vessels (jars), and thereby placing earthworms in the soil.

After 24 hours of acclimatization, the animals were rinsed in deionized water, dried on the filter paper and weighed. In order to confirm the homogeneity of earthworm culture, 20 specimens were weighed individually. Thereby the earthworms were weighed in groups of 10 specimens. The groups of 10 animals were randomly arranged to test vessels. Test vessels were placed under the experimental conditions.

On the 28th day of the experiment the contents of each jar were transferred to a clean, metal tray. Adults were removed from the artificial soil, and then counted and weighed in groups of 10 animals. Also, any symptoms of intoxication - behavioral and morphological changes – were noted. Artificial soil with cocoons was gently transferred back into appropriate jars and incubated under the same conditions for a further 4 weeks.

On the 56th day of experiment, juveniles were regained from the artificial soil. Animals and cocoons were retrieved by manually hand sorting the artificial soil. The entire contents of each jar were gently transferred to a clean, metal tray. Artificial soil was gradually sorted and young earthworms and cocoons were transferred to Petri dishes. Earthworms and cocoons were counted when the entire content of the single jar was sorted. Artificial soil from each of the test vessels was sorted twice in accordance with the OECD 222 Guideline.

In the current study the definitive test was performed as limit test including a single test item concentration - 1000 mg test item per 1 kg of dry weight of artificial soil and control. The test was carried out with 8 replicates for both – test item concentration and control.

2.Reference test

Physiological condition of earthworms was confirmed on the basis of the study with reference substance – carbendazim. According to OECD 222 significant effects should be observed between 1 – 5 mg/kg of the referenceitem. The study was performed with a single reference item concentration - 2,5 mg/kg of artificial soil (dry weight) and control. Both – reference item concentration and control consisted of 8 replicates, with 10 adult earthworms each.

3. Observations

During the experiment the following parameters were observed and recorded:

- Mortality, intoxication symptoms (unusual behavior like inability to dig into the soil, remaining motionless, morphological changes e.g. open wounds), in the each jar after 28 days. The earthworms were considered dead if they didn't respond for a gentile touching of the anterior end;
- Growth rate expressed as the average body weight on the basis of the measurements of weight of 10-speciments groups at the beginning and on the 28th day of the experiment;
- After 56 days the offspring number as well as (if possible) cocoons in the each jar. Additionally, the intoxication symptoms were assessed;
- Artificial soil pH value, water content at the beginning and at the end of the experiment in a single replicate of test item concentration and control
- The light intensity in a test room, directly above the test vessels
- The temperature during the study was measured continuously with a temperature recorder in the 1-hour intervals

4. Results analysis

The statistical data analysis was performed by ToxRat Professional software.

5. Deviations from the Guideline / Study Plan

Deviations from the Study Plan

On the 56th day of experiment, juveniles were regained from the artificial soil according to SPT-E/85 procedure and in accordance with OECD 222 Guideline. Due to the fact that the juvenile extraction method established in the study plan turned out to be time-consuming, it was changed to hand sorting of the soil. The primary method assumed that earthworms will be regained from the soil by the heating test vessels gradually from 40º C to 60 ºC in a water bath. Cocoons, which were not eaten or did not decomposed were retrieved from the soil (after removal of the young earthworms) by flushing the soil by water, first placing it on the mesh with a mesh diameter of 0,5 mm. During the current study earthworms and cocoons were retrieved by manually hand sorting the artificial soil. The entire contents of each jar were gently transferred to a clean, metal tray. Artificial soil was gradually sorted and young earthworms and cocoons were transferred to Petri dishes. Earthworms and cocoons were counted when the entire contents of the single jar was sorted. Artificial soil from each of the test vessels was sorted twice in accordance with the OECD 222 Guideline.

Deviations from the Guideline

On the 47th day of the experiment, the temperature had decreased below the lower limit value (18°C) for 0,10°C for a period of 8 hours, which was not in accordance with the OECD 222 Guideline and SPB-E/09 procedure. As the temperature decrease was slight and lasted for a short time compared to the total time of the experiment, it was indicated that it had no significant effect on the study and obtained final results.

Cross-section area of test vessels was smaller than recommended by guideline – approx. 120cm^2 in relation to recommended 200cm^2. Therefore, test item was incorporated into whole volume of artificial soil, not spread on the surface, earthworms were exposed for the test item same throughout the substrate volume. Therefore it was assumed that the deviation did not affect significantly the experiment and obtained results.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.4-1 Observations performed on adult earthworms on 28th day of experiment – definitive test

Observation time: 28th day				
Concentration [mg/kg]	Replicate	Number of alive	Number of dead	Intoxication symptoms
Control	1	10	0	no symptoms of intoxication
	2	10	0	
	3	10	0	
	4	10	0	
	5	10	0	
	6	10	0	
	7	10	0	
	8	10	0	
1000	1	10	0	no symptoms of intoxication
	2	10	0	
	3	10	0	
	4	10	0	
	5	10	0	
	6	10	0	
	7	10	0	
	8	10	0	

Table 9.4-2 Weights of 10 adults earthworms groups at the beginning and at the end of the experiment – definitive test

Concentration [mg/kg]	Replicate	Weight of 10 specimens: the beginning of the experiment [g]	Mean weight of the specimen: the beginning of the experiment [mg]	Mean weight for 10 specimens for the concentration – the beginning of the experiment [g]	The weight of the living specimens – after 28 days of the experiment [g]/(x) – living specimens	The mean weight of the specimen – after 28 days of experiment [mg]	Mean weight of living specimens for the concentration – after 28 of experiment [g]	Mean change in body weight of living specimens during 28 days of experiment [%]	Mean change in body weight of living specimens for concentration during 28 days of experiment [%]
Control	1	4,31	431	4,13	4,52	452	4,50	4,87	9,33
	2	3,93	393		4,44	444		12,98	
	3	4,08	408		4,66	466		14,22	
	4	4,97	497		5,21	521		4,83	
	5	4,23	423		4,42	442		4,49	
	6	3,90	390		4,26	426		9,23	
	7	3,67	367		4,27	427		16,35	
	8	3,92	392		4,22	422		7,65	
1000	1	4,10	410	3,89	4,42	442	4,24	7,80	9,08
	2	3,93	393		4,29	429		9,16	
	3	4,35	435		4,84	484		11,26	
	4	3,85	385		3,88	388		0,78	
	5	3,50	350		3,87	387		10,57	
	6	3,75	375		4,16	416		10,93	
	7	3,96	396		4,12	412		4,04	
	8	3,65	365		4,31	431		18,08	

Table 9.4-3 Statistics for the young earthworms obtained during the experiment*

Test item concentration	Control	1000 [mg/kg]
Mean offspring number	112,250	103,875
Standard deviation	9,8525	4,7037
The coefficient of variation of reproduction	8,8%	4,5%

* - calculated by ToxRat Professional

Table 9.4-4 Inhibition of adult earthworms growth and reproduction* - definitive test

Test item concentration	Inhibition of reproduction [%]	Inhibition of growth [%]	Mortality [%]
Control Mean	0,00	0,00	0,00
1000 [mg/kg]	7,46	5,86	0,00

* - calculated by ToxRat Professional

Table 9.4-5 Number of offspring earthworms and cocoons on 56th day of experiment – definitive study

Concentration [mg/kg]	Replicate	Number of offspring earthworms	Number of cocoons	Intoxication symptoms
Control	1	99	15	no symptoms of intoxication
	2	128	17	
	3	117	8	
	4	111	10	
	5	102	8	
	6	116	12	
	7	120	11	
	8	105	9	
1000	1	100	9	no symptoms of intoxication
	2	98	10	
	3	110	11	
	4	101	17	
	5	103	6	
	6	111	8	
	7	106	7	
	8	102	8	

Table 9.4-6 Weights of 10 adults earthworms groups at the beginning and at the end of the experiment – reference test

Concentration [mg/kg]	Replicate	Weight of 10 specimens – the beginning of the experiment [g]	Mean weight of the specimen - the beginning of the experiment [mg]	Mean weight for 10 specimens for the concentration – the beginning of the experiment [g]	The weight of the living specimens – after 28 days of the experiment [g]/(x) – living specimens	The mean weight of the specimen – after 28 days of experiment [mg]	Mean weight of living specimens for the concentration – after 28 of experiment [g]	Mean change in body weight of living specimens during 28 days of experiment [%]	Mean change in body weight of living specimens for concentration during 28 days of experiment [%]
Control	1	4,31	431	4,13	4,52	452	4,50	4,87	9,33
	2	3,93	393		4,44	444		12,98	
	3	4,08	408		4,66	466		14,22	
	4	4,97	497		5,21	521		4,83	
	5	4,23	423		4,42	442		4,49	
	6	3,90	390		4,26	426		9,23	
	7	3,67	367		4,27	427		16,35	
	8	3,92	392		4,22	422		7,65	
2,5	1	4,18	418	3,98	4,21	421	4,03	0,72	1,40
	2	3,98	398		4,03	403		1,26	
	3	4,35	435		4,41	441		1,38	
	4	3,80	380		3,92	392		3,16	
	5	3,74	374		3,81	381		1,87	
	6	3,91	391		3,94	394		0,77	
	7	3,72	372		3,77	377		1,34	
	8	4,12	412		4,15	415		0,73	

Table 9.4-7 Observations performed on adult earthworms on 28th day of experiment – reference test

Observation time: 28th day				
Concentration [mg/kg]	Concentration [mg/kg]	Concentration [mg/kg]	Concentration [mg/kg]	Concentration [mg/kg]
Control	1	10	0	no symptoms of intoxication
	2	10	0	
	3	10	0	
	4	10	0	
	5	10	0	
	6	10	0	
	7	10	0	
	8	10	0	
2,5	1	10	0	earthworms less lively compared to the control specimens; apathetic; visible small injuries and waists; earthworms smaller and thinner than those in control
	2	10	0	
	3	10	0	
	4	10	0	
	5	10	0	
	6	10	0	
	7	10	0	
	8	10	0	

Table 9.4-8 Number of offspring earthworms and cocoons on 56th day of experiment – reference test

Concentration [mg/kg]	Replicate	Number of offspring earthworms	Number of cocoons	Intoxication symptoms
Control	1	99	15	no symptoms of intoxication
	2	128	17	
	3	117	8	
	4	111	10	
	5	102	8	
	6	116	12	
	7	120	11	
	8	105	9	
2,5	1	28	3	earthworms clearly smaller and thinner, less lively compared to control; gut filled with food less, in comparison to the control specimens
	2	28	1	
	3	36	0	
	4	32	0	
	5	37	2	
	6	24	1	
	7	31	0	
	8	22	3	

Table 9.4-9 Inhibition of adult earthworms growth and reproduction * – reference test

Test item concentration	Inhibition of reproduction [%]	Inhibition of growth [%]	Mortality [%]
Control Mean	0,00	0,00	0,00
2,5 [mg/kg]	73,50	10,44	0,00

* - calculated by ToxRat Professional

B. OBSERVATIONS

The average offspring number in the control was 112,3 (minimal number 99, maximal number 128) and 103,9 in the test item concentration (minimal number 98, maximal number 111). The coefficient of variation of reproduction was 8,8% in the control and 4,5% in the test item concentration. The cocoons number in each control and test item concentration replicates was also recorded. No mortality of adult earthworms was observed in any replicate of test item concentration nor control. Also, no symptoms of intoxication neither behavioral and morphological changes were observed in both test item concentration and control. The total weight of groups of worms from each replicate was measured at the beginning and at the end of the experiment. There was no statically significant difference between the control group and the concentration of the test item 1000,00 mg/kg dry weight of artificial soil observed. In both – control and test item concentration the increase in body weight was observed. The mean growth rate for all replicates in control was 9,33% (minimal increase 4,49%, maximal increase 16,35%). The mean growth rate for all replicates in test item concentration was 9,08% (minimal increase 0,78%, maximal increase 18,08%).

III. CONCLUSIONS

Obtained study results and performed statistical calculations indicate, that test item – iron pyrophosphate, tested in it's maximal - according to OECD 222 – applicable concentration 1000mg/kg, has no effect on survival and growth (expressed as the change in body weight) of adult earthworms during the 4 weeks of experiment as well as reproduction expressed as young earthworms number at the end of the study. Test item shows no toxicity against earthworms *Eiseniafoetidafoetida*. Basing on the obtained results, the NOEC value (NOEC ≥ 1000mg/kg) for reproduction was calculated. Calculation of the other endpoints - EC_x, NOEC, LOEC, LD_{50}, was not possible due to mathematical reasons.

RMS comments:

The study of Earthworm Reproduction Test was performed in GLP conditions and according OECD TG 222. No significant deviations from the test guideline which could affect test results were noted. Since no effects were observed at the highest concentration in the range-finding test (i.e. 1000 mg/kg), the reproduction test was performed as a limit test, using a test concentration of 1000 mg/kg. The validity criteria of test were met. The study is considered valid and established endpoint may be used for risk assessment.

The exposure at a concentration of 1000 mg a.s. /kg for 28 days did not affect survival, behaviour and body weight of adult earthworms and caused slight inhibition of reproduction (7.5%). Mean numbers of juveniles produced in control group and 1000 mg/kg group were similar with the coefficient of variation of reproduction below 10%.

NOEC for mortality, body weight and reproduction : ≥ 1000 mg a.s. /kg d.w.soil

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

Besides the studies of effects of the BW01 GB effect on the soil-dwelling arthropods *Aleocharabilineata* and *Poeciluscupreus* and ferric pyrophosphate on earthworm and risk assessment for these species (see B.9.5.2. andB.9.4.1) a study on reproduction of predatory mite in soil was carried out.

B.9.4.2.1 Effects on survival and reproduction of Collembolan (*Folsomia candida*) in soil

Report: Rathke A. 2017, Collembolan (*Folsomia candida*) Reproduction Test in Soil

Study code: 161024BK / ICR17408

Guideline: OECD 232

GLP: Yes

Executive Summary:

The effects of Ferric pyrophosphate on the reproduction of *Folsomia candida*in artificial soil were determined in a laboratory study. The aim of the test was to determine the effects of the test item on mortality and reproduction of *Folsomia candida*in artificial soil (5 % peat) by cutaneous and alimentary uptake. The test item was incorporated into artificial soil once at experimental starting with the test item concentrations 16.3 - 29.4 - 52.9 - 95.3 - 171 - 309 - 556 - 1000 mg/kg soil dry weight (DW). As control artificial soil without test item was used. After application, ten juvenile female *Folsomia candida* (10 days old) were placed onto artificial soil of each replicate. 8 replicates for the control and 4 replicates for each test item concentration were tested. During exposure, springtails were fed with granulated dry yeast (without emulsifier). After 28 days, adult mortality and reproduction were assessed.
Under laboratory test conditions Ferric pyrophosphate had no statistically significant effects on the mortality and reproduction.
After exposure for 28 days no statistically significant effects in mortality and reproduction rate could be observed. Therefore, the NOEC for mortality and reproduction was determined to be ≥ 1000 mg/kg DW and the LOEC was determined to be > 1000 mg/kg DW. The LC_{20} and LC_{50} value for mortality and the EC_{10}, EC_{20} and EC_{50} values based on reproduction could not be calculated, but were all determined to be > 1000 mg test item/kg soil dry weight (the maximum rate tested).

I. MATERIALS AND METHODS

A. MATERIALS:

1**. Test Material:**Ferric pyrophosphate
Description: White to yellow powder
Batch No: 120327086
Purity: 101.73%
Storage: in hermetic container in dry, cool place

2. Vehicle: artificial soil
3. Test animals
Species: *Folsomia candida* WILLEM (Mandibulata, Antennata, Apterygota, Collembola)
Age: 10 days, female
Source: University Bremen
Acclimation period: at least 1 day
Diet: granulated yeast (without emulsifier)

4. Environmental conditions
Temperature: 20 ± 2°C
Humidity: soil moisture about 60 % WHC
Photoperiod: 400 - 800 lux, 16 h

B. STUDY DESIGN AND METHODS:

1. In life dates:2017-01-25 to 2017-02-22
2. Experimental treatments
Based on the results of the preliminary test 8 test item concentrations in geometrical series were tested as follows: 16.3 - 29.4 - 52.9 - 95.3 - 171 - 309 - 556 - 1000 mg Ferric pyrophosphate/kg DW (spacing factor 1.8). Artificial soil was treated once at experimental starting (day 0) with the test item. Juvenile springtails (10 days old) were added to the treated soil after application. 28 days after insertion of juvenile collembolans, mortality of adults and number of instars were determined.
3. Observations
Mortality of adults and number of instars, pH-value and soil moisture.
4. Results analysis
The statistical data analysis was performed by ToxRat and SigmaPlot software.
5. Deviations from the Guideline / Study Plan
None

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.4-10 Adult mortality [%] after 28 days of exposure

Test item [mg/kg DW]	Replicate								Mortality [%]	
	1	2	3	4	5	6	7	8	Mean ± SD	Corrected
16.3	10	0	20	40	---				17.5 ± 17.1	15.1
29.4	40	10	20	0					17.5 ± 17.1	15.1
52.9	20	0	10	10					10.0 ± 8.16	7.35
95.3	20	40	60	0					30.0 ± 25.8	27.9
171	20	20	0	20					15.0 ± 10.0	12.5
309	0	0	0	10					2.50 ± 5.00	-0.37
556	30	10	0	0					10.0 ± 14.1	7.35
1000	10	10	10	20					12.5 ± 5.00	9.92
Control	# (0)	10	0	10	0	0	0	0	2.86 ± 4.88	-

--- not determined

Outlier (reproduction data) after Dixon& Hartley (α=0.05), not taken into account

Table 9.4-11 Reproduction (number of juveniles) after 28 days of exposure

Test item [mg/kg DW]	Replicate								Mortality [%]		
	1	2	3	4	5	6	7	8	Mean ± SD	CV [%]	Inhibition to control [%]
16.3	884	737	534	624	---				695 ± 151	21.7	- 13.9
29.4	551	683	656	755					661 ± 84.6	12.8	-8.36
52.9	606	824	667	731					707 ± 93.2	13.2	-15.9
95.3	618	650	705	531					626 ± 72.8	11.6	-2.62
171	597	515	636	450					550 ± 83.3	15.2	9.84
309	575	616	645	655					623 ± 35.9	5.76	-2.13
556	533	570	571	654					582 ± 51.2	8.79	4.59
1000	793	643	566	538					635 ± 114	18.0	- 4.10
Control	# (1024)	557	655	549	533	678	571	728	610 ± 75.9	12.4	-

--- not determined

Outlier (reproduction data) after Dixon& Hartley (α=0.05), not taken into account

B. OBSERVATIONS

Mortality of Adults

The mortality of adults was determined after exposure for 28 days. At test end the control mortality was 2,86 %. No statistically significant effect between the control and the test item concentrations could be observed. The corrected mortality in the test item concentrations were between - 0.37 % (309 mg/kg DW) and 27.9 % (95.3 mg/kg DW).

Reproduction

The reproduction rate of the springtails was determined after exposure for 28 days. The mean reproduction rate of the control was 610 juveniles and between 550 (171 mg/kg DW) and 707 (52.9 mg/kg DW) juveniles in the test item treatments. A statistically significant reduction of the reproduction rate compared to the control could not be observed.

III. CONCLUSIONS

Under laboratory test conditions Ferric pyrophosphate had no statistically significant effects on the mortality reproduction. After exposure for 28 days no statistically significant effects in mortality and reproduction rate could be observed. Therefore, the NOEC for mortality and reproduction was determined to be ≥ 1000 mg/kg DW and the LOEC was determined to be > 1000 mg/kg DW. The LC_{20} and LC_{50} value for mortality and the EC_{10}, EC_{20} and EC_{50} values based on reproduction could not be calculated, but were all determined to be > 1000 mg test item/kg soil dry weight (the maximum rate tested).

RMS comments:
The effects of ferric pyrophosphate on *Folsomia candida* was performed in GLP conditions and according OECD 232Collembolan Reproduction Test in Soil. No significant deviations from the test guideline were noted. All validity criteria were met. No mortality nor effects on reproduction were observed after dosing of test item in doses 16.3; 29.4; 52.9; 95.3; 171; 309; 556 and 1000 mg/kg soil dry weight .The study is considered valid and established endpoints may be used for risk assessment.
The study is considered acceptable with following endpoint:
28 days $NOEC_{mortality/reproduction}$ ≥1000 mg test item/kg soil dry weight
LOEC was determined to be > 1000 mg/kg DW
LC_{20} and LC_{50} value for mortality and the EC_{10}, EC_{20} and EC_{50} values based on reproduction could not be calculated, but were all determined to be > 1000 mg test item/kg soil dry weight (the maximum rate tested).

B.9.4.2.2 Effects on survival and reproduction of predatory mite in soil

Report: Parsons C. 2017, Ferric pyrophosphate - A laboratory test to determine the effects of fresh residues on the predatory soil mite *Hypoaspisaculeifer* (Acari, Laelapidae)
Study code: BROS-16-2
Guideline: OECD 226
GLP: Yes

Executive Summary:

The test item in this study was an unformulated sample of Ferric Pyrophosphate. The aim was to determine under laboratory test conditions whether Ferric Pyrophosphate has harmful effects on the predatory soil mite *Hypoaspisaculeifer* (Canestrini) (Acari, Laelapidae).
Based on the numbers of offspring produced, the 14-day $EC5_0$ and the EC_{20} and EC_{10} values for Ferric Pyrophosphate were all estimated as > 1000 mg test item/kg soil dry weight. The LC_{50} was also estimated to be > 1000 mg test item/kg soil dry weight. The overall NOEC with respect to both mite survival and reproduction was 1000 mg test item/kg soil dry weight, the maximum concentration evaluated.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Ferric pyrophosphate
Description: white to pale yellow powder
Batch No: 120327086
Purity: 101.73%
Storage: ambient laboratory conditions

2. Vehicle: artificial soil with 5 % peat content

3. Test animals

Species: *Hypoaspisaculeifer*
Age: 30 and 31 days after start of egg laying
Source: culture maintained at the Test Facility
Acclimation period: not applicable
Diet: cheese mites and juvenile springtails

4. Environmental conditions

Temperature: 19.6 - 20.5°C
Humidity: no data
Photoperiod: 16 h, 480 - 640 lux

B. STUDY DESIGN AND METHODS:

1. In life dates: range find test - application on 18 January 2017, definitive test - application on 16 February 2017

2. Experimental treatments

A preliminary range-finding test was carried out with five concentrations of the test item and a water control. The endpoints were an assessment of the condition of the introduced mites at 14 days and an assessment of their reproduction, i.e. the numbers of F1 progeny present 14 days after treatment (DAT). For the range-finding test, there were two replicate arenas per treatment, each set up with 10 adult female mites (i.e. a total of 20 mites per treatment).

A definitive test was then carried out using one treatment concentration of the test item (equivalent to 1000 mg test item/kg soil dry weight) and an untreated control treatment. Since the test item has a low solubility in water and acetone, it was applied to the artificial soil using a sand carrier. Adult female mites were introduced into replicated containers of treated soil and the endpoint was an assessment of any changes in the mite population at 14 days, with the numbers of both adult and juvenile mites being recorded. For the definitive test, there were eight replicate arenas for the control treatment and for the test-item treatment. Each was set up with 10 adult female mites (i.e. a total of 80 mites per treatment).

3. Observations

In both the range-finding and definitive tests, the number of both surviving adults and of F1 progeny (i.e. juvenile test mites) in each test arena were assessed at 14 DAT.

4. Results analysis

The following statistical tests were used:

- Fisher's Exact Test
- Mann-Whitney U-test
- Abbott's formula

5. Deviations from the Guideline/ Study Plan

None

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.4-12 The effects of Ferric Pyrophosphate on the survival and reproduction of *Hypoaspisaculeifer*

Treatment	Concentration (mg test item/ kg soil dry wt.)	% mortality at 14 DAT a)	Corrected % mortality at 14 DAT b)	Mean no. progeny per replicate c)	% change in numbers of progeny, relative to control d)
Control	-	4	-	231	-
Ferric pyrophosphate	1000	3	-1	237	-3

a) Mortality amongst mites originally introduced. Treatments were compared by Fisher's Exact Test (α = 0.05), but there were no significant differences.

b) Derived using Abbott's formula. A negative number represents an increase in mite survival, relative to the control.
c) The treatment was compared to the control using Mann-Whitney U-test ($\alpha = 0.05$), but there were no significant differences.
d) A negative value indicates an increase in reproduction, relative to the control.

III. CONCLUSIONS

In this 14-day laboratory test with Ferric Pyrophosphate and the predatory soil mite *Hypoaspisaculeifer*, the NOEC with respect to assessments of both adult mite survival and reproduction was 1000 mg test item/kg soil dry weight, the maximum concentration evaluated. The 14-day EC_{50}, EC_{20} and EC_{10} values for effects on reproduction were all estimated to be > 1000 mg test item/kg soil dry weight. The LC_{50} was also estimated to be > 1000 mg test item/kg soil dry weight.

RMS comments:
The effects of ferric pyrophosphate on the predatory soil mite *Hypoaspisaculeifer* (Acari, Laelapidae) was performed in GLP conditions and according OECD 226 Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil. No significant deviations from the test guideline were noted. All validity criteria were met. No mortality nor effects on reproduction were observed after dosing of test item in doses 1000, 100, 10, 1 and 0.1 mg test item/kg soil dry weight.The study is considered valid and established endpoints may be used for risk assessment.
The study is considered acceptable with following endpoints:
14 day EC_{50}: > 1000 mg /kg soil dry weight
$NOEC_{mortality/reproduction}$-1000 mg /kg soil dry weight

B.9.5 Effects on Soil Nitrogen Transformation

Based on point 4 of the introduction to section 8 of the Commission Regulation (EU) No. 283/2013 setting out the data requirements for active substances, a study of the effect on nitrogen transformation in soil was performed using the representative plant protection product BW01 GB instead of the active substance as manufactured.

B.9.6 Effects on Terrestrial Non-target Higher Plants

B.9.6.1 Summary of screening data

The plant protection product containing ferric pyrophosphate does not display a herbicidal effect nor does it regulate plant growth, nor does it affect non-target land plants in any way. In the field efficiency studies conducted, the molluscicide investigated did not cause any symptoms of phytotoxic effects in crops (winter wheat and winter oilseed rape) and ornamental plants (hosta and marigold). Ferric pyrophosphate is an inorganic salt, whose components - iron and phosphorus - occur naturally in all aquatic and terrestrial ecosystems. These elements are nutrients indispensable for proper growth and development of plants and animals, and are naturally absorbed by plants from soil. Therefore, no adverse effects are to be expected after the application of the PPP containing ferric pyrophosphate.

B.9.6.2 Testing on non-target plants

Ferric pyrophosphate is an inorganic salt, whose components - iron and phosphorus - occur naturally in all aquatic and terrestrial ecosystems. These elements are nutrients indispensable for proper growth and development of plants and animals, and are naturally absorbed by plants from soil. Therefore, no adverse effects are to be expected after the application of the PPP containing ferric pyrophosphate. Thus, further studies on non-target plants are not required.

B.9.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

BW01 GB plant protection product is applied as granular bait, which minimizes the possibility of exposure of non-target terrestrial organisms. Moreover, ferric pyrophosphate is a substance Generally Recognized As Safe by the US FDA. It is used as a dietary supplement to prevent and treat iron-deficiency anemia and as an additive to infant cereals, breakfast cereals and chocolate-drink powders. In fact, both iron and phosphorus occur naturally in food because they are an inherent element of plant and animal metabolism. Iron is a micronutrient and phosphorus is a macronutrient, both play a key role in the growth and development of plants. Iron is indispensable for the

development of chloroplasts and is a component of cytochromes. Phosphorus, on the other hand, is necessary for the formation of high-energy compounds such as ATP and is a component of nucleic acids and several key coenzymes. Thus, it is believed that there is no need to conduct further studies on the effect of ferric pyrophosphate on other terrestrial organisms.

B.9.8 Effects on Biological Methods for Sewage Treatment

Given the natural occurrence of iron and phosphorus in the terrestrial and aquatic environments, and the lack of risk to soil micro- and macro-organisms, it is believed that the use of the BW01 GB plant protection product containing ferric pyrophosphate will not affect biological methods for waste treatment and will not have negative effects on sewage-treatment plants, thus a study of the effects on biological methods for waste treatment is not required.

B.9.9 Monitoring Data

There exists no data on monitoring that could be directly related to negative impact of ferric pyrophosphate on non-target organisms.

B.9.10 Biological activity of metabolites potentially occurring in ground water

Not relevant

B.9.11 References Relied On

Reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y / N	Owner
Vol. 3. B.9 B.9.1.1.1	[redacted]	2013	Ferric Pyrophosphate: Avian Acute Oral Toxicity Study in Japanese Quail Study code: P/13559/AAOT/13 OECD Guideline No. 223 GLP: Yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
Vol. 3. B.9 B.9.1.1.3	[redacted]	2015	Ferric Pyrophosphate Avian Reproduction Test (Coturnixcoturnix japonica) Study code: G/33/14 Guideline: OECD Guideline No. 206 (1984) GLP: YES Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.1.2.1	[redacted]	2013	FERRIC PYROPHOSPHATE: Acute Oral Toxicity Study on Rats – Fixed Dose Method Study code: PO-2/13 Guideline: OECD Guideline No. 420 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.2.1	[redacted]	2013	Fish Acute Toxicity Test according to OECD Guideline No 203, Study code: 0003/0024/E Guideline: OECD 203 GLP: yes / Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.

B. 9.2.4.1	Ziółkowska, A Wickiel, G.	2015	Daphnia sp. Acute Immobilization Test according to OECD Guideline No 202 Study code: 0003/0022/E Guideline: OECD No 202 GLP: yes / Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.2.6	Heisterkamp I.	2015	Alga Growth Inhibition Test according to OECD 201 (23 March 2006) Study code: 1040 Guideline: OECD 201 GLP: yes / Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k..
B.9.3.1.1.1	Ziółkowska, A	2013	Honeybees, Acute Oral Toxicity Test according to OECD 213 Study code: 0003/0026/E Guideline: OECD 213 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.3.1.1.2	Winkler, J.	2013	Honeybees, Acute Contact Toxicity Test according to OECD 214 Study code: 0003/0025/E Guideline: OECD 214 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.4.1	Winkler, J.	2014	Earthworm reproduction test according to OECD 222 Study code: 0003/0028/E Guideline: OECD TG 222 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.4.2.1	Rathke, A.	2017	Collembolan (Folsomia candida) Reproduction Test in Soil , study code: 161024BK / ICR17408, 26.04.2017 Guideline: OECD 232 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.4.2.2	Parsons, CH	2017	Ferric Pyrophosphate – A laboratory test to determine the effects of fresh residues on the predatory soil mite Hypoaspisaculeifer (Acari, Laelapidae) Study code: BROS-16-2 Guideline: OECD 226 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.

Appendix 1

Literature search on Ferric pyrophosphate Prepared by Jim Shine, Kerona Scientific Ltd. on behalf of Bros Spółka z o.o. March 2019

1. INTRODUCTION

This report summarises the results of a scientific peer-review open literature search conducted in order to support the authorization of the active substance ferric pyrophosphate under Regulation (EU) No 1107/2009.

Ferric pyrophosphate acts as a molluscicide and is intended for the control of slugs and snails in all edible and inedible plants. It has not yet been approved as an active substance in plant protection products and is to be regarded as a new active substance according to the regulation.

Article 8(5) of Regulation (EU) No 1107/2009 requires that a scientific peer-reviewed open literature search must be conducted on the active substance, dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier.

The literature search was conducted in accordance with the EFSA guidance on *Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009* (EFSA Journal 2011;9(2):2092). In order to capture all data requirements of interest, a single-concept search strategy was adopted, using search terms for the active substance and its synonyms only, without additional concepts.

The main categories of data requirements on chemical active substances are:

- Toxicological and toxicokinetic studies
- Metabolism and residue data
- Fate and behaviour in the environment
- Ecotoxicological studies
- Other data requirements for which information may have a direct or indirect effect on overall risk assessment.

The literature review should also address endocrine disruption.

2. CRITERIA FOR RELEVANCE

Publications were deemed relevant if they provided information on ferric pyrophosphate that addressed the data requirements on chemical active substances.

The relevance criteria are presented in Table 1 below.

Table 1: Relevance criteria

Data requirement indicated by the corresponding OECD data point number	**Criteria for relevance**
Toxicological and metabolism studies (OECD IIA 5.1 to 5.7)	1. Well-defined test material 2. Test species relevant to the mammalian toxicological assessment 3. Number of animals per group sufficient to establish statistical significance 4. Several dose levels tested (at least 3), preferably including a negative control, to establish a dose-response 5. Well-described test methodology 6. Description of the observations, mortality etc., and chemical analysis performed
Residues (OECD IIA 6)	1. Well-defined test material 2. Any information on residues in crops relevant to the EU 3. Well-described test methodology and results
Fate and behaviour in the environment (OECD IIA 7)	1. Any information that could affect the environmental parameters, degradation profile or endpoints used in the risk assessment 2. Any environmental monitoring information relevant to the usage pattern 3. Well-defined test material 4. Well-described test methodology
Ecotoxicological studies (OECD IIA 8)	1. Well-defined test material 2. Relevant test species 3. Number of test organisms per group sufficient to establish statistical significance 4. Several dose levels tested, preferably including a negative control 5. Well-described test methodology 6. Description of the observations, mortality etc., and chemical analysis performed
Other data requirements for which information may have a direct or indirect on the overall risk assessment, including endocrine disruption	1. Well-defined test material 2. Any information that could affect the risk assessment other than under the OECD data points already addressed

3. SEARCH METHODS AND INITIAL RESULTS

The scientific literature was searched using the following resources:

- Scopus
- Web of Science
- Embase
- Reaxys
- Agricola

The justification for the choice of each database is provided in Table 2.

The EFSA guidance states that the search strategy should capture literature published during the 10 years prior to the dossier submission date, as required by Article 8(5) of Regulation (EC) No. 1107/2009. The date span of the search was 2005 to 2015, as the dossier for ferric pyrophosphate was submitted for approval in December 2015.

A single-concept search strategy was adopted, using search terms for the active substance and its synonyms only, without additional concepts. The search terms chosen were “ferric pyrophosphate” and “iron pyrophosphate” as well as the CAS number 10058-44-3. Given the range of the data requirements to be addressed, the alternative strategy of multiple focused searches would have required an excessive number of search terms to cover all possibilities. It was assumed that the single-concept strategy would produce a large number of irrelevant documents, but it was decided that this approach would be less time-consuming as well as highly sensitive.

Table 2: Reporting of the search process for scientific peer-reviewed open literature in bibliographic databases

Data requirement(s) captured in the search	**Details of the searches**				
Ferric pyrophosphate – covers all data requirements	**Scopus**	**Web of Science Core Collection**	**Embase**	**Reaxys**	**Agricola**
	Justification for choosing the source: Large multi-disciplinary database of research literature, with over 16,000 titles and over 69 million records.	**Justification for choosing the source:** Large multi-disciplinary database, with over 20,000 journals and 1.4 billion cited references.	**Justification for choosing the source:** Covers the most important international biomedical literature. Over 8,500 journals and >32 million records. Includes Medline.	**Justification for choosing the source:** Covers over 16,000 chemistry-related periodicals.	**Justification for choosing the source:** Publications and resources encompassing all aspects of agriculture and related disciplines.
	Date of the search: 13/03/2019	**Date of the search:** 13/03/2019	**Date of the search:** 13/03/2019	**Date of the search:** 13/03/2019	**Date of the search:** 13/03/2019
	Date span of the search: 2005-2015	**Date span of the search:** 2005-2015	**Date span of the search:** 2005-2015	**Date span of the search:** 2005-2015	**Date span of the search:** 2005-2015
	Date of the latest database update included in the search:				
	Updated daily.	Updated daily.	Updated daily.	Updated weekly.	Updated daily.
	Search strategies used for this data requirement:				
	(ALL ("ferric pyrophosphate") OR ALL ("iron pyrophosphate") OR ALL ("10058-44-3")) AND (PUBYEAR > 2004 AND PUBYEAR < 2016)	ALL=("ferric pyrophosphate" OR "iron pyrophosphate" OR "10058-44-3") Timespan: 2005-2015.	('ferric pyrophosphate' OR 'iron pyrophosphate' OR '10058-44-3') AND [2005-2015]/py	(Chemical Name is 'Ferric pyrophosphate' OR Chemical Name is 'Iron pyrophosphate' OR CAS Registry Number is 10058-44-3) AND (Publication Year >=2005 AND Publication Year <=2015)	Keyword Anywhere(ferric pyrophosphate) OR Keyword Anywhere(iron pyrophosphate) OR Keyword Anywhere(10058-44-3)(DATE=2005-2015)
	Total number of summary records retrieved:				
	742	179	115	61	44
	Total number of summary records retrieved after removing duplicates: 802				

4. RESULTS OF STUDY SELECTION PROCESS

Following the initial search, a total of 802 summary records (excluding duplicates) were identified. These were screened for their likely relevance by rapid assessment of titles and, where available, abstracts.

A total of 799 summary records were found to be obviously irrelevant and were excluded from further consideration. It must be noted, however, that although these records did not directly address the listed data points or fulfil the relevance criteria, a substantial number were found by assessment of abstracts to provide information on the use of ferric pyrophosphate as a supplement in food to address iron deficiency, indicating that the substance is safe in the context of human health.

The 3 remaining summary records that were potentially relevant were assessed in detail by reading the full-text documents. Following this assessment, all 3 records were excluded from further consideration.

The results of the different stages of the study selection process are presented in Table 3. The full-text documents that were deemed irrelevant and excluded from further consideration are listed in Table 4.

Table 3: Results of the study selection process, for each data requirement or group of data requirements searched

Data requirement(s) captured in the search (as indicated in Table 2)	**n**
Total number of summary records retrieved after all searches of peer-reviewed literature (excluding duplicates)	802
Number of summary records excluded from the search results after rapid assessment for relevance	799
Total number of full-text documents assessed in detail	3
Number of studies excluded from further consideration after detailed assessment for relevance	3
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

Table 6: Report of the studies excluded after detailed assessment of full-text documents

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier
Li, Bo; Brett, Michael T.	2013	The influence of dissolved phosphorus molecular form on recalcitrance and bioavailability	Environmental Pollution 2013;182:37-44	Does not cover any OECD data point and/or does not fulfil the relevance criteria
Lobo A.R., Cocato M.L., de Sá L.R.M., Colli C.	2014	Dietary iron overload: Short- and long-term effects on cecal morphometry in growing rats	Journal of Nutritional Science and Vitaminology 2014;60(6):397-402	Does not cover any OECD data point and/or does not fulfil the relevance criteria
Sturm B., Ternes N., Goldenberg H., Scheiber-Mojdehkar B.	2009	Ferric pyrophosphate: Biochemical characterisation and metabolic fate in HepG2 cells	American Journal of Hematology 2009;84(8):E287	Does not cover any OECD data point and/or does not fulfil the relevance criteria

5. CONCLUSIONS

A literature search was conducted to identify scientific peer-reviewed open literature relating to ferric pyrophosphate to support the authorization of this active substance under Regulation (EU) No 1107/2009. Several bibliographic databases were searched, and the date span of the search was 2005 to 2015.

A total of 802 unique summary records were identified, and a rapid assessment of titles and, where available, abstracts was conducted. On assessment, 799 of these records were deemed to be irrelevant and excluded from further consideration. The full text of the remaining 3 records was assessed to determine both relevance and reliability, after which all records were excluded. Therefore no literature of direct relevance to the required data points was identified, although a considerable amount of literature was identified describing the use of ferric pyrophosphate as an iron supplement in the human diet, implying that the substance is safe in the context of human health.