Ferric pyrophosphate

Volume 3

Product data: VITROL GB (BW01 GB)

Annex B.9: Ecotoxicology

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B.9 ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES

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 m^2). 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 toxicity to birds

Based on the acute toxicity data on the active substance presented in point B.9.1.1.1 and the results of acute toxicity study on mammals (B.9.1.2.1), it is predicted that the product BW01 GB will not demonstrate acute toxicity to birds.

B.9.1.1.2 Higher tier data on birds

The results of studies on mammals show a lack of toxicity both for the active substance and the PPP, thus dietary toxicity of BW01 GB to birds was not investigated. The use of the PPP containing ferric pyrophosphate does not pose any risk to birds, thus higher-tier studies with the use of birds are not necessary

B.9.1.2 Effects on terrestrial vertebrates other than birds

B.9.1.2.1 Acute oral toxicity to mammals

Report: [redacted]2014, Acute Oral Toxicity – Fixed Dose Procedure

Study code: 0001/0104/T

Guideline: OECD 420

GLP: yes

Executive Summary:

The test method consisted in an administration of test material doses to test animal groups according to step wise procedure. The aim of the study was evaluation of acute toxicity and health consequences after single dose intake. A dose level for main study was determined based on sighting study.

After single dose test material application in dose of 300 mg/kg b.w. no changes were observed. The animal survived the whole period of study. After single dose test material application in dose of 2000 mg/kg b.w. for next four animals in main study, no symptoms of toxicity were observed. All animals survived whole period of study.

During 14 days of observation, a body weight gain were noted except one animal from sighting study (2000 mg/kg b.w. dose group) of which the body weight did not change. Based on lack of clinical changes and positive results of an autopsy (no macroscopic pathological changes of examined organs) it is concluded that lack of the body weight gain was not caused by test material BW01 GB. There were no changes in post-mortem examination in all six test animals. Based on the results, the test material BW01 GB is classified to Category 5/Unclassified – according to GHS classification system - according to OECD 420 and is not classified in accordance with Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 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.

1. MATERIALS AND METHODS

A. MATERIALS

1. Name: BW01 GB

Test material description: turquoise granules

Batch no.: 032014-P82

Name of active ingredient: Iron (III) pyrophosphate

Content of active ingerdient: 3% (nominal content)

Storage conditions: temperature: 10 - 30ºC, humidity: 30 - 70%

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

3. Test animals

Species: Wistar rat

Age: 10 - 11 weeks

Weight: 178-208 g

Source: [redacted]

Acclimation period: 5 days

Diet: laboratory feed 'Labofeed B' from animal feed manufacturer 'Morawski' and filtered tap water ad libitum

4. Environmental conditions

Temperature: 22ºC ± 3ºC

Humidity: minimum 30%, maximum 70%

Lightening: artificial, the sequence being 12 hours light, 12 hours dark (12/12)

The exchange of indoor air: approx. 15 times / h

Cages size: (transparent cages, made of polycarbonate) 54 x 39 x 21 cm

Animals housed: 1,1 and 4 in cage

B. STUDY DESIGN AND METHODS:

1. In life dates: 22.04.14-12.05.14

2. Experimental treatments

Preliminary test

The first step of the experiment was the sighting study in which suspension of test material in 0,5% methylcellulose solution was administrated in dose 300 and 2000 mg/kg b.w. with 48 hours break between.

Definitive test

Based on the results from the sighting study, the main study dose was 2000 mg/kg b.w. The test material in fixed dose was administrated for rest four animals in group (altogether five animals received a dose of 2000 mg/kg of test material). The test substance was administered in a single dose by gavage using a stomach tube for all animals after 17 – 18 hours of starving.

3. Observations

After test material administration test animals were observed for 14 days. During the experiment, general and detailed observation were provided. Detailed clinical observation were performed during first 30 minutes after dosing, i.e. after 10, 20, 30 min. and after 1, 2, 3, 4 hours and once a day for 14 days. Results of detailed clinical observations of animals were noted. Detailed clinical observation included changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. During whole observation period, general condition of the animals was controlled once a day and evaluated of morbidity, signs of toxicity and mortality. In day of dose administration and after 7 and 14 days the weight of each animal were measured. After 14 day of observation the animals were sacrificed and postmortem examined. Postmortem examination included external surface of the body, all orifices, cranial, thoracic and abdominal cavity together with its contents examination and detailed macroscopic examination.

4. Deviations to the Guideline

No changes and variations of the Guideline.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.1-1 Summary of the results

Dose (mg/kg b.w.)	Number of animals	Number of deaths	Clinical observations	Results of macroscopic examination during gross necropsy
300	1	0	No clinical changes	No macroscopic changes
2000	1	0	No clinical changes	No macroscopic changes
2000	4	0	No clinical changes	No macroscopic changes
GHS Category = 5/unclassifieed				

Table 9.1-2 Animal body weight

Number of rat	Dose [mg/kg b.w.]	Body weight [g]			Weight in day of sacrifice	Body weight gain
		Day of experiment				
		0	7	14		
19/14/1	300	208	225	231	231	23
20/14/1	2000	195	195	198	198	3
21/14/1	2000	196	220	228	228	32
22/14/1	2000	189	203	234	234	45
23/14/1	2000	178	199	205	205	27
24/14/1	2000	178	201	214	214	36

B. OBSERVATIONS

After test material administration in single dose of 300 mg/kg b.w. animal did not show the signs of toxicity. The animal survived the whole period of the study (14 days).

After test material administration in single dose of 2000 mg/kg b.w. animal did not show the signs of toxicity. The animal survived the whole period of the study (14 days).

After single dose test material application in dose of 2000 mg/kg b.w. to next four animals in the main study, no symptoms of toxicity were observed. All animals survived whole period of study (14 days). During 14 days of observation, a body weight gain were noted except one animal from sighting study (2000 mg/kg b.w. dose group) of which the body weight did not change. Based on lack of clinical changes and positive results of an autopsy (no macroscopic pathological changes of examined organs) it is concluded that lack of the body weight gain was not caused by test material BW01 GB.

After 14 days of observation the gross necropsy was performed. The gross necropsy (included a detailed external examination, as well as all the holes and cavities and detailed macroscopic examination of organs in the cranial cavity, thoracic and abdominal) did not show macroscopic changes during general visual inspection of animal body and examination of internal organs in all cases. The organs were correct structure and size, and on the cross-section, the structure of organs was also correct. There were no deviations that would suggest acute toxic effects of the test material BW01 GB.

III. CONCLUSIONS

Based on the results, the test material BQ01 GB is classified to Category 5/Unclassified – according to GHS classification system - according to OECD 420 and is not classified in accordance with Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 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} of BW01 GB for rats is above 2000 mg/kg bw. It is noted that toxicity of product is not higher than that of active substance.

The result (endpoint) may be used for risk assessment.

B.9.1.2.2 Higher tier data on mammals

The use of the PPP containing ferric pyrophosphate does not pose any risk to mammals, thus higher-tier studies with the use of mammals are not necessary.

B.9.1.2.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Based on the conducted studies of acute and reproductive toxicity to birds and acute toxicity to mammals, as well as literature data on the use of ferric pyrophosphate as a dietary supplement and food additive, and the 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 terrestrial vertebrates.

B.9.2 Risk Assessment for Birds and Other Terrestrial Vertebrates

B.9.2.1 Risk assessment for birds

Summary

Effects on birds for BW01 GB were not evaluated as part of the EU review of Ferric pyrophosphate. However, further data on BW01 GB is not relevant as active substance data on toxicity to birds is used and additional formulation data are not considered essential. Risk assessments for BW01 GB with the proposed use pattern are provided here and are considered adequate.
The risk assessment for effects on birds is carried out for the recommended scenarios for granular applications according to EFSA Journal (2009)[1] and is considered adequate.

BW01 GB is a molluscicide in granule form applied up to 6 times with a 14 day interval with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in oilseed rape, with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in winter wheat and with up to 50 kg/ha (corresponding to 1.5 kg a.s./ha) in ornamentals (worst case).

The acute risks of BW01 GB to birds were assessed in first tier from ratios between toxicity endpoints, estimated from studies with Ferric pyrophosphate and the maximum oral exposure following application according to the proposed worst-case use pattern.
The TER values presented in Table 1.1-2 indicate risk to birds. However, due to the absence of acute toxicity in available vertebrate studies as well as the nature of the active substance and its role as dietary supplement and food additive, the acute risk is considered acceptable.

Both components of BW01 GB - ferric and phosphate ions are naturally occurring in the environment, e.g. in soil and plant material as well as Ferric pyrophosphate is approved to be added to food sources. Additionally, Ferric pyrophosphate, due to its very low solubility in water and lipids as well as poor bioavailability, has no potential for accumulation under normal physiological conditions. Thus, no risk assessment for long-term toxicity is required.

The risk of secondary poisoning is considered negligible as Ferric pyrophosphate is practically insoluble. In addition, there is no risk for birds resulting from exposure to contaminated drinking water.

The use of BW01 GB does not pose unacceptable risk to birds.

1 European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Table 1-1 Effects on birds, Ecotoxicological endpoints for birds

Substance	Species	DAR Ferric pyrophosphate, 2017	Endpoints used in risk assessment
Acute			
Ferric pyrophosphate	*Coturnix japonica*	LD_{50} > 2000 mg a.s./kg bw ~~LD_{50} > 3228 mg a.s./kg bw[a]~~	Extrapolated LD_{50} from a limit dose = 3228 mg/kg bw[a]
Chronic			
Ferric pyrophosphate	*Coturnix japonica*	NOEC ≥ 111.2 mg a.s./kg bw/day (NOEC ≥ 1000 mg/kg fodder)	-

[a] According to EFSA Journal (2009; page 16)[2], it is permissible to extrapolate an LD_{50} value upwards in cases where there is no mortality or a single mortality at a limit dose in an acute avian toxicity study. In the acute toxicity study on Japanese quail, the acute oral LD_{50} was greater than 2000 mg a.s./kg bw based on the absence of mortality at the highest dose tested of 2000 mg/kg bw. An extrapolation factor of 1.614 based on 5 animals tested, was used to calculate the extrapolated LD_{50} of 3228 mg a.s./kg bw.

Table 1-2 Most critical toxicity/exposure ratios (TERs) for birds ingesting granules of BW01 GB

	Acute risk assessment
Single application rate (worst case) [g a.s./ha]	1500
Toxicity Endpoint [mg a.s./kg bw]	LD_{50} = 3228
Birds ingesting granules as a source of food	
ETE [mg a.s./kg bw]	10 800
TER (Trigger)	**> 0.30 (10)**
Birds ingesting granules with/as grit	
DGritD [mg a.s./kg bw][a]	910.0[b] / 1146.3[c]
TER (Trigger)	**> 3.5[b] (10) / > 2.8[c] (10)**
Birds ingesting granules when seeking seeds as food	
DGD [mg a.s./kg bw]	214.6[b] / 265.7[c]
TER (Trigger)	> 15.0[b] **(10)** / > 12.1[c] **(10)**
Birds ingesting granules when eating soil-contaminated food	
DDSD [mg a.s./kg bw]	0.42
TER (Trigger)	> 7686 **(10)**
Remark: These TER values are not considered as realistic and relevant. The calculation of the TER_A values relies on the maximum dose tested for acute toxicity, i.e. 2000 mg a.s./kg bw. It has to be taken into consideration that no effects have been observed at this dose level. Furthermore, both ferric and phosphate ions are natural constituents of soils serving as essential nutrients in animal and plant physiology with no potential for accumulation under normal physiological conditions. Ferric pyrophosphate is also approved to be added to food sources.	

TER values in bold indicate that risk cannot be excluded.

[2]European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

a	For BW01 GB only the exposure scenario for large granules is to be considered since granule size of BW01 GB is 3 - 4 mm.
b	Calculation based on following assumptions: weight/granule = 15 mg; loading = 0.45 mg a.s.; density = 333.3 granules/m²
c	Calculation based on following assumptions: weight/granule = 20 mg; loading = 0.6 mg a.s.; density = 250 granules/m²
ETE	Estimated theoretical exposure
DGritD	Daily grit dose
DGD	Daily granule dose
DDSD	Daily dry soil dose
TER	Toxicity exposure ratio

Toxicity

The avian toxicity endpoints for Ferric pyrophosphate that are most appropriate for acute ecological risk assessment are summarised in Table 1-3 below. For full details of the studies performed with Ferric pyrophosphate, please refer to the study reports. No toxicity was observed in these studies up to the highest doses tested. Furthermore, no acute toxicity was observed in rats up to a dose of 2000 mg a.s./kg body weight. Thus, the toxicity of Ferric pyrophosphate and BW01 GB is considered to be low and the use of a LD_{50} > 2000 mg a.s./kg body weight obtained for Japanese quail is considered to be overprotective.

Both components of BW01 GB - ferric and phosphate ions are naturally occurring in the environment, e.g. in soil and plant material as well as Ferric pyrophosphate is approved to be added to food sources. Additionally, Ferric pyrophosphate, due to its very low solubility in water and lipids as well as poor bioavailability, has no potential for accumulation under normal physiological conditions. Due to the general toxicological profile, no long-term toxicity is expected.

Table 1-3 Summary of avian toxicity endpoints for Ferric pyrophosphate

Study type	Substance	Species	Endpoint	Value [mg a.s./kg bw]	Reference
Acute oral toxicity	Ferric pyrophosphate	Japanese quail (*Coturnix japonica*)	LD_{50}	> 2000	Mhaske, 2013
Long-term toxicity and reproduction	Ferric pyrophosphate	Japanese quail (*Coturnix japonica*)	NOEC	≥ 111.2	Wróbel, 2015

Exposure

BW01 GB is a molluscicide containing the active substance Ferric pyrophosphate. It is applied as slug granules up to 6 times with a 14 day interval with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in oilseed rape, with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in winter wheat and with up to 50 kg/ha (corresponding to 1.5 kg a.s./ha) in ornamentals (worst case).

According to EFSA Journal (2009)[3], birds may be exposed to granular formulations in different ways:

a) Birds may ingest granules as a source of food.

b) Birds may ingest granules as grit.

c) Birds may mistake granules for small seed.

d) Birds may ingest granules when they eat food contaminated with soil.

e) Birds may consume food contaminated with residues resulting from granular applications (slug-eating birds).

Since it can be assumed that a second to fifth application is only performed when granules of the previous application have been consumed by slugs or otherwise disappeared, no multiple application factor (MAF) was considered in the risk assessment for birds ingesting granules of BW01 GB (exposure paths a to d). No MAF

[3] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

is incorporated into the risk assessment for slug-eating birds (exposure paths e) since slugs usually feed only once at a BW01 GB granule, immediately after the application granules are available to excess and die thereafter. Therefore, it can be expected, that no residues will build up and that the maximum values after the second application are not higher than after the first application.

Acute toxicity exposure ratio (TER_A)

Acute risk was calculated using the acute LD_{50} value for the active substance based on the study performed with Ferric pyrophosphate and the extrapolation factor of 1.614, due to the absence of mortality at the tested dose of 2000 mg/kg bw. A TER_A value below 10 indicates a potential acute risk to birds. The results are presented in **Table 1.1-1** to **Table 1.1-4**.

First-Tier risk assessment

a) Birds ingesting granules of BW01 GB as a source of food

Since there is a possibility that birds will mistake granules for food, a risk assessment is conducted based on the calorific value of the granular material.

According to the EFSA Journal (2009)[4] the species of concern for the first-Tier assessment is an omnivorous bird (e.g. house sparrow with a body weight of 27.7 g). The BW01 GB granules have a caloric value of 328.75 kcal/100 g (corresponding to 13.76 kJ/g), which is comparable to cereals seeds and might substitute this food type in the diet of the indicator species.

The estimates of the food intake rate (FIR) of a certain food type are based on means of daily energy expenditure of the indicator species, energy and moisture content and assimilation efficiencies of the respective food type. The FIR can be calculated as follows:

$$FIR = \frac{DEE}{FE \times \left(1 - \frac{MC}{100}\right) \times \frac{AE}{100}} \left[\text{g fresh weight/d}\right]$$

With:		
DEE	=	Daily energy expenditure of the indicator species (calculated according to EFSA Journal (2009), Appendix G)
FE	=	Food energy [kJ/g d.w.]
MC	=	Moisture content [%]
AE	=	Assimilation efficiency [%]

The calculation of risk for birds ingesting granules of BW01 GB as a source of food were performed in line with Appendix G of the Guidance (EFSA Journal 2009; 7(12):1438).

DEE was calculated based on default values given in Table 2 of Appendix G for "passerines'

log DEE= log a + b + log bw

log DEE = 1,032 + 0,676 x 1,44

log DEE = 2,01

DEE = 101,65

4 European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

FIR = 101,65 / (13,76*(1-7/100)*(80/100)) = 9,9

FIR/bw = 9,9/27,7 = 0,36

ETE = 0,36 * 30000 * 1 = 10800

TER = > 3228/10800 = > 0,3

Since the performed calculation is a first-tier, the worst case scenario was assumed in which PT=1.

The estimated theoretical exposure (ETE), i.e. the uptake of a compound via a single food item is given by the following equation:

$$ETE = \frac{FIR}{bw} \times C \times PT \left[mg/kg\ bw/d \right]$$

With:		
FIR	=	Food intake rate of indicator species [g fresh weight/d]
bw	=	Body weight [g]
C	=	Concentration of compound in fresh diet [30000 mg a.s./kg]
PT	=	Fraction of diet obtained in treated area (number between 0 and 1)

The TER value was calculated by dividing the acute endpoint (LD_{50}) by the estimated daily exposure (ETE):

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw/day)}{ETE\ (mg/kg\ bw/day)}$$

The assessment is shown in **1.1-1.**

Table 1.1-1 Acute risk assessment for birds ingesting granules of BW01 GB as a source of food

Indicator species	bw [g]	DEE [kJ]	Food type	FE [kJ/g d.w.]	MC [%]	AE* [%]	FIR [g/d]	FIR/ bw	C [mg a.s./kg]	ETE [mg/ kg bw]	LD_{50} [mg/ kg bw]	TER_A (10)
Omnivorous bird (House sparrow)	27.7	101.65	Cereals seeds	13.76	7	80	9.9	0.36	30000	10800	3228	**0.30**

DEE Daily energy expenditure of the indicator species
FE Food energy (please refer to the confidential document on the formulation content of BW01 GB)
MC Moisture content (please refer to the confidential document on the formulation content of BW01 GB)
AE Assimilation efficiency
FIR Food intake rate of indicator species
C Concentration of compound in fresh diet
ETE Estimated theoretical exposure
TER Toxicity exposure ratio

* value from EFSA Journal (2009)[5], Appendix G

The TER_A value for birds ingesting granules as a source of food is below 10, indicating a potential acute risk.

However, the calculation of the TER_A value relies on the maximum dose tested for acute toxicity, i.e. 2000 mg a.s./kg bw considering the extrapolation factor of 1.614. It has to be taken into account that no effects (no mortality, no clinical signs, no effect on the body weight gain, no effects on food intake) have been observed at the tested dose level and the use of a NOEL instead of a LD_{50} increases the conservatism of the risk assessment. Furthermore, testing higher doses in order to obtain a definite LD_{50} exceeding 2000 mg/kg bw, is not recommended in the test guideline. As a further refinement options either an avoidance study in pens or a field study is relevant for granular formulations. Notwithstanding, due to animal welfare further vertebrate testing is not considered appropriate.

Furthermore, both components of BW01 GB - ferric and phosphate ions are natural constituents of soils serving as essential nutrients in animal and plant physiology with no potential for accumulation under normal physiological conditions. Iron is the second most abundant metal in the environment and the fourth most abundant element, building around 5.6% of the Earth's crust. The content and distribution of iron in different soil types varies but typically it is in the average range of 1 – 5% (10 – 50 g/kg). It is an essential nutrient that is required for various biochemical and physiological functions. In animal physiology it participates in electron transfer, DNA synthesis, oxygen transport and other cellular functions. Inadequate supply of this micro-nutrient results in a variety of deficiency diseases and syndromes. Also, phosphorus is an element essential for the functioning of every cell. It is a component of many important compounds such as nucleic acids, numerous coenzymes, ADP and ATP, a key compound in intracellular energy transfer. As iron, it is a natural occurring element of the terrestrial environment (appearing in concentrations of 0.01 - 0.2 %).

Bearing the above in mind, the amount of iron (III) and phosphate added by the application of BW01 GB is by several orders of magnitude smaller than the natural content commonly found in soils. Additionally, iron and, in particular, phosphate are applied in considerable amounts to agricultural soils in fertilizers.

Additionally, due to the very low solubility of Ferric pyrophosphate in water and lipids, the bioavailability and absorption in the body of organisms is low (2% of an average absorption of iron from food fortified). Therefore, Ferric pyrophosphate does not possess potential for accumulation in organisms under normal physiological conditions.

Furthermore, a risk of bioaccumulation is not expected based on the function as dietary supplement in food sources. Ferric pyrophosphate has been approved as a safe source of iron added to food, even for young children and infants, processed cereal-based food and food for children, food for special medical purposes as well as total diet replacement (Regulation (EU) No 609/2013[6]). Ferric pyrophosphate is also included on the list of vitamins and mineral substances which may be used in the manufacture of food supplements according to Commission Regulation (EC) No 1170/2009[7].

Considering all the above-mentioned arguments, effects due to consumption of granules of BW01 GB can be excluded and are not further assessed.

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5 European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

6 Regulation (EU) no 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. Official Journal of the European Union. Off. J. Eur. Union 29.6.2013, L181, 35-56.

7 Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. Off. J. Eur. Union 1.12.2009, L314, 36-42.

RMS: Birds ingesting granules of BW01 GB as a source of food
The estimated theoretical exposure (ETE) of indicative bird to ferric pyrophosphate ingesting granules of BW01 GB containing 2.4% of ferric pyrophosphate as a single food item (worst case scenario) was calculated according to formulas and default values given in appendix G of the Guidance document (EFSA Journal 2009; 7 (120: 1438). The ETE amounts to 10 800 mg of ferric pyrophosphate/kg bw with assumption that all the energy comes from a diet composed of BW01 GB granules, which is a worst case scenario rather improbable in real life. There is no data to assess a preference of birds to use granules of BW01 GB as a food item, therefore the appropriate fraction of diet obtained in treated area cannot be set.
This estimated theoretical exposure (ETE) is much higher that a dose of 2000mg/kg bw applied in the acute toxicity limit test in birds, which did not induced mortality or clinical symptoms of acute intoxication. TER_{ac} calculated for acute single exposure using the extrapolated LD50 from a limit dose of 3228mg/kg equals to 0.3. The interpretation of TERac for acute risk assessment creates high uncertainty , because real value of LD50 of ferric pyrophosphate for birds might be much higher than 3228mg/kg equals. For example, the LD50 for ferric phosphate has been estimated to be above 5000 mg/kg bw (EFSA Journal 2015;13(1):3973).

This first-tier risk assessment pointing out on the unacceptable risk of acute toxic effects in omnivorous and granivorous birds, but it is overconservative due to:

- lack of the acute toxic effects produced by ferric pyrophosphate observed in the acceptable acute oral toxicity study in birds
- lack of real, oral LD_{50} for birds and for mammals which might be much higher than the extrapolated LD50
- overestimation of ferric pyrophosphate consumption with granules of BW01 GB as a source of food

The ferric pyrophosphate did not induce any symptoms of acute toxicity in birds and mammals at the highest doses foreseen in the acceptable methods for regulatory assessment of acute toxicity. Therefore, the use of TER_{ac} for acute risk assessment posed by ferric pyrophosphate seem to be misleading and is linked with a high uncertainty since it indicate the existence of risk of acute poisoning of birds, while a substance is known of not having acute toxicity.

b) Birds ingesting granules of BW01 GB with/as grit

According to EFSA Journal (2009)[8], grit consumption by farmland birds is an important constituent of dietary intake both for mineral content and mastication. Therefore, birds might be exposed to granules of BW01 GB when ingesting them with/as grit.

The acute daily grit dose ($DGritD_{acute}$) for small and large granules is calculated according to the following equations:

According to section 5.1.6 of EFSA Guidance document small granules have a size between 0.75 and 2 mm, and large granules size between 2 and 6 mm. BW01 GB is distributed as granules with size 2 - 3 mm, so the whole product is considered to be in large granules

$$DGritD_{acute}(\text{small granules}) = 651 \times \frac{G_{density}}{15200 + G_{density}} \times G_{loading}$$

$$DGritD_{acute}(\text{large granules}) = 2453 \times \frac{G_{density}}{71 + G_{density}} \times G_{loading}$$

With:		
$G_{density}$	=	Number of granules on soil surface
$G_{loading}$	=	Amount of the active substance in one granule

[8] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

For BW01 GB only the exposure scenario for large granules is to be considered since granule size of BW01 GB is 2 - 3 mm.

With respect to the range of granule size given, calculations were performed based on two different average assumptions for granule weights, considering the worst-case scenario for the use in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha. Thus, the assessments for the other uses in ornamentals with the application rate of 25 kg product/ha, and in winter wheat and oilseed rape are considered as covered.

- An average granule weight of 15 mg/unit results in a $G_{density}$ of 333.3 units/m² and a $G_{loading}$ of 0.45 mg a.s./unit.
- An average granule weight of 20 mg/unit results in a $G_{density}$ of 250 units/m² and a $G_{loading}$ of 0.6 mg a.s./unit.

The TER value was calculated by dividing the acute endpoint (LD_{50}) by the acute daily grit dose ($DGritD_{acute}$) for large granules:

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw/day)}{DGritD_{acute}\ (mg/kg\ bw/day)}$$

The assessment for the worst-case use in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha is shown in **Table 1.1-2**. The assessment for the uses in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha covers also the other uses with the application rate of 25 kg product/ha in ornamentals, winter wheat and oilseed rape.

Table 1.1-2 Acute risk assessment for birds ingesting granules of BW01 GB with/as grit (worst-case, ornamentals with an application rate of 50 kg product/ha)

Crops	Single application rate [g a.s./ha]	$G_{density}$ [granules/m²]	$G_{loading}$ [mg a.s./granule]	LD_{50} [mg a.s./kg bw]	$DGritD_{acute}$ [mg/kg bw]		TER_A (10)	
					Small granules	Large granules	Small granules	Large granules
Ornamentals (Hosta, Tagetes)	1500	333.3	0.45	> 3228	Not applicable*	910.0	Not applicable*	**> 3.5**
		250	0.6			1146.3		**> 2.8**

*For BW01 GB, only the exposure scenario for large granules is to be considered since granule size of BW01 GB is 2 - 3 mm.
$G_{density}$ Number of granules on soil surface
$G_{loading}$ Amount of the active substance in one granule
$DGritD_{acute}$ acute daily grit dose
TER Toxicity exposure ratio

The TER_A values for birds ingesting granules with/as grit are below 10, indicating that a refined acute risk assessment is required. Due to the relatively high application rates for the other uses in ornamentals, winter wheat and oilseed rape, also for these the trigger value of 10 cannot be met.

However, the calculation of the TER_A values relies on the maximum dose tested for acute toxicity, i.e. 2000 mg a.s./kg bw, considering the extrapolation factor of 1.614. It has to be taken into account that no effects (no mortality, no clinical signs, no effect on the body weight gain, no effects on food intake) have been observed at the tested dose level and the use of a NOEL instead of an LD_{50} increases the conservatism of the risk assessment. Furthermore, the formula proposed by EFSA Journal (2009)[9], for the risk assessment of granules taken for grit and the guideline for testing acute toxicity seem not to be harmonised. Even for a LD_{50} > 2000 mg/kg bw, (corresponding to the maximum accepted test rate according to the OECD guideline 223) the

[9] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

resulting TER_A of < 10 would still indicate a potential risk. Testing higher doses in order to obtain a definite LD_{50} exceeding 2000 mg/kg bw, is not recommended in the test guideline. As a further refinement options either an avoidance study in pens or a field study is relevant for granular formulations. Notwithstanding, due to animal welfare further vertebrate testing is not considered appropriate.

Furthermore, both components of BW01 GB - ferric and phosphate ions are natural constituents of soils serving as essential nutrients in animal and plant physiology with no potential for accumulation under normal physiological conditions. Iron is the second most abundant metal in the environment and the fourth most abundant element, building around 5.6% of the Earth's crust. The content and distribution of iron in different soil types varies but typically it is in the average range of 1 – 5% (10 – 50 g/kg). It is an essential nutrient that is required for various biochemical and physiological functions. In animal physiology it participates in electron transfer, DNA synthesis, oxygen transport and other cellular functions. Inadequate supply of this micro-nutrient results in a variety of deficiency diseases and syndromes. Also, phosphorus is an element essential for the functioning of every cell. It is a component of many important compounds such as nucleic acids, numerous coenzymes, ADP and ATP, a key compound in intracellular energy transfer. As iron, it is a natural occurring element of the terrestrial environment (appearing in concentrations of 0.01 - 0.2 %).

Bearing the above in mind, the amount of iron (III) and phosphate added by the application of BW01 GB is by several orders of magnitude smaller than the natural content commonly found in soils. Additionally, iron and, in particular, phosphate are applied in considerable amounts to agricultural soils in fertilizers.

Additionally, due to the very low solubility of Ferric pyrophosphate in water and lipids the bioavailability and absorption in the body of organisms is low (2% of an average absorption of iron from food fortified). Therefore, Ferric pyrophosphate does not possess potential for accumulation in organisms under normal physiological conditions.

Furthermore, a risk of bioaccumulation is not expected based on the function as dietary supplement in food sources. Ferric pyrophosphate has been approved as a safe source of iron added to food, even for young children and infants, processed cereal-based food and food for children, food for special medical purposes as well as total diet replacement (Regulation (EU) No 609/2013[10]). Ferric pyrophosphate is also included on the list of vitamins and mineral substances which may be used in the manufacture of food supplements according to Commission Regulation (EC) No 1170/2009[11].

Considering all the above-mentioned arguments, effects due to consumption of granules of BW01 GB can be excluded and are not further assessed.

RMS: Birds ingesting granules of BW01 GB with/as grit
The acute risk for birds ingesting granules of BW01 GB with/as grit has been assessed in line with recommendation of the EU guidance document (Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. The calculated values of TER_A were found to be below 10, thus the risk refinement is required. However, this first-tier risk assessment pointing out on the unacceptable risk of acute toxic effects in granivorous birds seem to be overconservative and inappropriate due to lack of the acute toxicity of ferric pyrophosphate observed in the acceptable studies in birds and mammals. In fact, ferric pyrophosphate at the highest doses foreseen in the acceptable methods for assessment of acute toxicity did not demonstrate any symptoms of acute toxicity in birds and mammals. Therefore, calculation of TER_{acute} for acute risk assessment

10 Regulation (EU) no 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. Official Journal of the European Union. Off. J. Eur. Union 29.6.2013, L181, 35-56.

11 Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. Off. J. Eur. Union 1.12.2009, L314, 36-42.

might be misleading and creates a high uncertainty since it indicate the existence of risk of acute poisoning, while a substance is known of not having acute toxicity.

c) Birds ingesting granules of BW01 GB when seeking seeds as food

Granules of BW01 GB might be mistaken for weed seeds by seed-eating birds.

The acute daily granule dose (DGD_{acute}) is calculated according to the following equation:

$$DGD_{acute} = 620 \times \frac{G_{density}}{100 + G_{density}} \times G_{loading}$$

With:		
$G_{density}$	=	Number of granules on soil surface
$G_{loading}$	=	Amount of the active substance in one granule

With respect to the range of granule size given, calculations were performed with two different average assumptions for granule weights, considering the worst-case scenario for the use in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha. Thus, the assessment for the other uses in ornamentals with the application rate of 25 kg product/ha, winter wheat and oilseed rape is considered as covered.

- An average granule weight of 15 mg/unit results in a $G_{density}$ of 333.3 units/m² and a $G_{loading}$ of 0.45 mg a.s./unit.
- An average granule weight of 20 mg/unit results in a $G_{density}$ of 250 units/m² and a $G_{loading}$ of 0.6 mg a.s./unit.

The TERacute value was calculated by dividing the acute endpoint (LD_{50}) by the acute daily granule dose (DGD_{acute}):

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw/day)}{DGD_{acute}\ (mg/kg\ bw/day)}$$

The assessment for the worst-case use in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha is shown in **1.1-3**. The assessment for the uses in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha covers also the uses in ornamentals with the application rate of 25 kg product/ha, winter wheat and oilseed rape.

Table 1.1-3 Acute risk assessment for birds ingesting granules of BW01 GB when seeking seeds as food (worst-case, ornamentals with an application rate of 50 kg product/ha)

Crops	Single application rate [g a.s./ha]	$G_{density}$ [granules/m²]	$G_{loading}$ [mg a.s./ granule]	LD_{50} [mg a.s./kg bw]	DGD_{acute} [mg/kg bw]	TER_A (10)
Ornamentals (Hosta, Tagetes)	1500	333.3	0.45	> 3228	214.6[a]	> 15.0
		250	0.6		265.7[b]	> 12.1

$G_{density}$ Number of granules on soil surface
$G_{loading}$ Amount of the active substance in one granule
DGD_{acute} acute daily granule dose

TER Toxicity exposure ratio

a	Calculation based on following assumptions: weight/granule = 15 mg; loading = 0.45 mg a.s.; density = 333.3 granules/m²
b	Calculation based on following assumptions: weight/granule = 20 mg; loading = 0.6 mg a.s.; density = 250 granules/m²

The TER_A values for birds ingesting granules when seeking seeds as food are above 10, indicating that a refined acute risk assessment is not required.

RMS: Birds ingesting granules of BW01 GB when seeking seeds as food
Acute risk
The acute risk for birds ingesting granules of BW01 GB when seeking seeds as food has been assessed in line with recommendation of the EU guidance document (Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. The acute daily granule dose (DGDacute) for a small granivorous bird calculated according to formula and assumptions given in section 5.1.3. Birds ingesting granules when seeking seeds as food of EFSA guidance document amounted to 214.6 - 265.7 mg/kg bw, giving Toxicity Exposure Ration (TER of 15 - 12.1). This first-tier assessment that application of BW01 GB in accordance to GAP proposed by the applicant does not create a risk of acute toxic effects for granivorous birds.

d) Birds ingesting granules of BW01 GB when eating soil-contaminated food

Birds might be exposed to BW01 GB granules as part of ingested soil when seeking food.

The acute daily dry soil dose ($DDSD_{acute}$) is calculated according to the following equation:

$$DDSD_{acute} = 0.283 \times dosage\,[kg\ a.s./ha]$$

Dosage of BW01 GB following worst-case application in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha, is 1.50 kg a.s./ha. With the worst-case highest dosage, the dosages for the other uses in ornamentals with the application rate of 25 kg product/ha, winter wheat and oilseed rape are considered as covered.

The TER value was calculated by dividing the acute endpoint (LD_{50}) by the acute daily dry soil dose ($DDSD_{acute}$):

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw/day)}{DDSD_{acute}\ (mg/kg\ bw/day)}$$

The assessment for the worst-case use in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha is shown in **1.1-4**. The assessment for the uses in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha covers also the uses in ornamentals with the application rate of 25 kg product/ha, winter wheat and oilseed rape.

Table 1.1-4 Acute risk assessment for birds ingesting granules of BW01 GB when eating soil-contaminated food (worst-case, ornamentals with an application rate of 50 kg product/ha)

Crops	Dosage [kg a.s./ha]	LD_{50} [mg/kg bw]	$DDSD_{acute}$ [mg/kg bw]	TER_A (10)
Ornamentals (Hosta, Tagetes)	1.50	> 3228	0.42	> 7686

$DDSD_{acute}$ acute daily dry soil dose
TER Toxicity exposure ratio

The TER_A value for birds ingesting granules when eating soil-contaminated food is above 10, indicating that a refined acute risk assessment is not required.

RMS: Animals ingesting granules when eating soil-contaminated food

The acute risk for birds ingesting granules of BW01 GB when eating soil-contaminated food has been assessed in line with recommendation of the EU guidance document (Guidance Document on Risk Assessment for Birds & Mammals, EFSA Journal 2009; 7(12): 1438. The acute daily dry soil dose (DDSDac) for a small omnivorous bird calculated according to formula and assumptions given in section 5.1.4. *Animals ingesting granules when eating soil-contaminated food* of EFSA guidance document amounted to 0.42 mg/kg bw, giving Toxicity Exposure Ration TER of 7686, thus above a target value of 10. This first-tier assessment indicates that application of BW01 GB in accordance to GAP proposed by the applicant does not create a risk of acute toxic effects for omnivorous birds ingesting granules when eating soil-contaminated food.

e) Birds consuming other food items with residues from granular application of BW01 GB (slug-eating birds)

Due to the use of BW01 GB as slug granules, residues in other food items but slugs are not to be expected.

Ferric pyrophosphate is not a systemic substance and up-take via seedlings is not expected. Bioaccumulation and biomagnification in terrestrial food chains is not expected since Ferric pyrophosphate is virtually insoluble and there is no potential for accumulation under normal physiological conditions. However, an additional exposure scenario results from the use of BW01 GB as slug granule since birds may consume slugs containing residues of Ferric pyrophosphate.

At present, no standardized risk assessment scheme is available for the exposure scenario of slug eating birds. There is no data of possible contents of Ferric pyrophosphate in slugs after consumption of BW01 GB available; also, there is no reliable information on possible consumption of contaminated slugs by birds.

Ferric pyrophosphate affects the calcium metabolism of slugs with the result of a disturbance of the fluid household in turn with a disruption of feeding and mucus production. After consumption of BW01 GB the slugs retreat themselves and die in hidden places. Therefore, a consumption of slugs containing residues of Ferric pyrophosphate is very unlikely, as these are not achievable for birds after consuming BW01 GB and moving to hidden places.

Considering the above-mentioned arguments, effects due to consumption of slugs containing residues of Ferric pyrophosphate can be excluded and are not further assessed.

RMS. The probability of consumption by slug-eating birds of other food items with residues from granular application of BW01 GB is negligible. The justification provided by the applicant is acceptable.

1.2 Short and long-term toxicity exposure ratio (TER_{ST})

Short-term toxicity exposure ratio (TER_{ST})

Since Ferric pyrophosphate is considered to be not toxic to birds and furthermore does not have potential for accumulation, an addition of toxic effects cannot be expected. According to EFSA Journal (2009)[12], no assessment is required.

Long-term toxicity exposure ratio (TER_{LT})

BW01 GB can be regarded as safe for birds due to the natural occurrence of its both components - ferric and phosphate ions in the environment, the occurrence of Ferric pyrophosphate as the dietary supplementation in food sources and the general toxicological properties e.g. Ferric pyrophosphate has no potential for accumulation under normal physiological conditions.

It must also be considered that there were no acute effects at all at the highest dose tested (2000 mg a.s./kg bw) in Japanese quails. Also, the avian reproduction study conducted with Japanese quails excluded harmful effect of the active substance on reproduction and long-term toxicity up to the highest tested concentration (1000 mg Ferric pyrophosphate/kg fodder corresponding to 111.2 mg/kg b.w./day).

As a matter of fact, BW01 GB is applied when slug infestation occurs. Only when the applied product is eaten up by slugs, a second application is recommended. Thus, continuous long-term exposure of birds to the product is not expected.

RMS: Reproductive risk assessment

Calculation of the daily granule dose (DGD_{repro}) for small granivorous bird for reproductive risk assessment

$$\mathrm{DGD_{repro}} = 620 \times \frac{G_{\mathrm{density}}}{100 + G_{\mathrm{density}}} \times G_{\mathrm{loading}}$$

Depending upon $G_{density}$ the daily granule dose (DGD_{repro}) will be equal to 214.6 mg/kg bw/d or 265.7 mg/kg bw/d

$$\mathrm{TER}_{repro} = \frac{\mathrm{NOEC\ (mg/kg\ bw/day)}}{\mathrm{DGD_{repro}\ (mg/kg\ bw/day)}}$$

$$\mathrm{TER}_{repro} = \frac{>111.2\ \mathrm{mg\ a.s./kg\ b.w./day}}{265.7\ \mathrm{mg/kg\ bw}} = > 0.42$$

The TER_{repro} values for birds ingesting granules when seeking seeds as food (> 0.42) are below 5, although a real value for TER_{repro} cannot be established since TER was calculated using a value of NOEC (no observed effect concentration) obtained in the study in which the maximum treatment level used was ≥ 1000 mg Ferric pyrophosphate /kg fodder (i.e. ≥ 111.2 mg Ferric pyrophosphate /kg b.w./day) and this maximum treatment level did not produce any adverse effects on the parameters of reproduction evaluated during the experiment. Therefore, the real value of $NOEC_{repro}$ might be considerably higher, even higher than 2000mg/kg bw/day, since no acute toxicity of Ferric pyrophosphate was observed in birds and mammals. Since Ferric pyrophosphate in the light of existing data does not meet criteria for reproductive toxicity, it is questionable whether the estimation of reproductive risk for substance non-hazardous for reproduction is appropriate, taking into account that is probable that $NOEC_{repro}$ might be much higher than a top dose in a study for reproductive toxicity for birds.
Taking into account that iron and phosphate ions are ubiquitous in the environment, and that ferric pyrophosphate is used as the dietary supplementation and that ferric pyrophosphate did not produce symptoms of acute or chronic intoxication or effect on reproduction in acceptable studies it is concluded that BW01 GB does not create acute, long-term or reproductive risk for birds due to its application with a use pattern proposed in GAP.

[12] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

1.3 Baits: Concentration of active substance in bait in mg/kg

The concentration of Ferric pyrophosphate is 30000 mg /kg.

1.4 Pellets, granules, prills or treated seed

Amount of active substance in or on each item

With respect to the range of granule size given, calculations were performed with two different average assumptions for granule weights:

- A theoretical granule weight of 15 mg/unit results in a $G_{loading}$ of 0.45 mg a.s./unit.
- A theoretical granule weight of 20 mg/unit results in a $G_{loading}$g of 0.6 mg a.s./unit.

Proportion of active substance LD_{50} per 100 items and per gram of items

With respect to the range of granule size given, calculations were performed with two different average assumptions for granule weights:

- An average granule weight of 15 mg/unit results in a $G_{loading}$ of 0.45 mg a.s./unit. This assumption results in a nominal amount of 45 mg Ferric pyrophosphate/100 granules. This is equivalent to 2.25% of the LD_{50} of > 2000 mg a.s./kg bw.
- An average granule weight of 20 mg/unit results in a $G_{loading}$ of 0.6 mg a.s./unit. This assumption results in a nominal amount of 60 mg Ferric pyrophosphate/100 granules. This is equivalent to 3% of the LD_{50} of > 2000 mg a.s./kg bw.
- Both assumptions result in a nominal amount of 30 mg Ferric pyrophosphate/g BW01 GB. This is equivalent to 1.5% of the LD_{50} of > 2000 mg a.s./kg bw.

Table 1.4.2-1 Proportion of LD_{50} for Ferric pyrophosphate

LD_{50} (acute)	> 2000 mg a.s./kg bw	
Application amount	30 mg a.s./g granules*	
Amount of a.s. per g granule	0.45 mg a.s.	0.6 mg a.s.
Mean weight of granule	15 mg	20 mg
Mean amount of a.s. per 100 granules	45 mg a.s.	60 mg a.s.
Proportion of LD_{50} on 100 granules	2.25%	3%
Proportion of LD_{50} per g of granules	1.5%	

* nominal application rate

1.5 Effects of secondary poisoning

According to EFSA 2009[13], substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of bioaccumulation in terrestrial food chains and uptake through contaminated water.

The log P_{OW} of Ferric pyrophosphate cannot be estimated since Ferric pyrophosphate is practically insoluble in water. Furthermore, a risk of bioaccumulation is not expected based on the natural occurrence of both components of BW01 GB - ferric and phosphate ions in the environment and the absence of potential for accumulation under normal physiological conditions. Both, iron as well as phosphate is essential components in natural food sources.

Considering the above-mentioned arguments, effects of secondary poisoning can be excluded and are not further assessed.

[13] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

As for birds, risk assessment for plant protection products in the form of granules is usually conducted quantitatively to obtain a risk factor expressed as for example the ratio between toxicity and exposure (TER). However, the EFSA Guidance Document on Risk Assessments for Birds and Mammals (2009) points to numerous difficulties in the assessment of bird exposure to PPP in the form of granules. Thus, a weight-of evidence approach was considered more appropriate in the risk assessment for birds due to the following reasons:

- elements which are the components of ferric pyrophosphate - iron and phosphorus - occur naturally in the environment, and their function in plants and animals and the effects of their deficiency are well known
- neither ferric pyrophosphate nor the representative BW01 GB formulation display toxicity to birds and mammals
- the active substance is insoluble in water and organic compounds, and will be absorbed only to a limited extent, which means that it will be only partly bioavailable
- bioaccumulation can be ruled out
- ferric pyrophosphate is used as a dietary supplement and food additive
- iron and phosphate ions are intrinsic element of plant and animal metabolism

Study of acute toxicity after oral administration of ferric pyrophosphate in the Japanese quail (B.9.1.1.1) did not demonstrate mortality or any other toxicity symptoms in the birds for the highest dose tested i.e. 2000 mg/kg bw. Weight gain in the birds that were administered ferric pyrophosphate was comparable to weight gain in the control group. Similarly to the control group, no negative effects on food ingestion were observed in the birds that were administered the compound investigated. The conducted study on the effect of ferric pyrophosphate on reproduction of the Japanese quail (B.9.1.1.3) demonstrated that the material investigated, used in the concentration of 1000 mg/kg bw, did not cause bird mortality or any macroscopic changes, did not affect the amount of feed ingested or body weight of the birds and did not affect reproduction parameters assessed in the experiment. Based on the studies of the active substance, it might be predicted that the PPP containing 3% ferric pyrophosphate will not be toxic to birds.

Since ferric pyrophosphate is insoluble in water, octanol/water partition coefficient cannot be established. However, the risk of bioaccumulation can be ruled out due to the natural occurrence of iron and phosphorus in the environment, the key role of these elements in the metabolism of plants and animals, and the use of ferric pyrophosphate as a dietary supplement and food additive.

Based on the information above, it is believed that the use of the PPP containing ferric pyrophosphate is unlikely to be of any consequence for birds. Given the available data on toxicity and properties of ferric pyrophosphate, the risk to birds resulting from the use of BW01 GB in accordance with the rules of good agricultural practice and label instructions is acceptable. Long-term exposure of birds to the granules is considered negligible.

B.9.2.2 Risk assessment for other terrestrial vertebrates

In the study of acute oral toxicity to mammals for the active substance (B.9.1.2.1) and for the PPP (B.9.1.2.1), no toxicity symptoms were observed for the highest dose tested. After a single administration of the material investigated in the dose of 2000 mg/kg bw, no clinical symptoms were observed in any of the animals, all of them survived, weight gain was observed and there were no pathological changes. Given the results of toxicological

studies and the use of ferric pyrophosphate as a dietary supplement and food additive, as well as the occurrence of iron and phosphorus in all living organisms, it was considered unnecessary to conduct risk analysis in accordance with relevant risk factor analysis. It can be stated that the end-points obtained from the toxicological studies for birds and mammals, and publicly available information on ferric pyrophosphate indicate lack of risk to mammals resulting from the exposure to the PPP containing ferric pyrophosphate used in accordance with the rules of good agricultural practice and label instructions. Ferric pyrophosphate is non-toxic to terrestrial vertebrates and the risk resulting from the use of the PPP containing this active substance in accordance with the rules of good agricultural practice and label instructions is acceptable.

Overview and summary

Table 2-1 Effects on terrestrial vertebrates other than birds, Ecotoxicological endpoints for mammals

Substance	Species	DAR Ferric pyrophosphate, 2017	Endpoints used in risk assessment
Acute			
Ferric pyrophosphate	Rat	LD_{50} > 2000 mg a.s./kg bw	LD_{50} > 2000 mg a.s./kg bw
Chronic			
Ferric pyrophosphate	Rat	NOAEL > 1000 mg a.s./kg bw	-

Summary

The effects on terrestrial vertebrates other than birds for BW01 GB were not evaluated as part of the EU review of Ferric pyrophosphate. However, further data on BW01 GB is not relevant as active substance data on toxicity to terrestrial vertebrates other than birds is used and additional formulation data are not considered essential. Risk assessments for BW01 GB with the proposed use pattern are provided here and are considered adequate.

The risk assessment for effects on vertebrates other than birds is carried out according to EFSA Journal (2009)[14].

BW01 GB is a molluscicide intended to be used as slug granules up to 6 times with a 14 day interval with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in oilseed rape, with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in winter wheat and with up to 50 kg/ha (corresponding to 1.5 kg a.s./ha) in ornamentals (worst case).

The potential exposure of terrestrial vertebrates other than birds to BW01 GB was estimated following 6 applications of BW01 GB at 50 kg product/ha (worst case).

The acute risks of BW01 GB to wild mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with Ferric pyrophosphate, and the daily intake.

The Tier-1 TER values were calculated for the recommended scenarios for granular formulations according to EFSA (2009). The results are presented in **Table 2-2**.

[14] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Table 2-2 Most critical toxicity/exposure ratios for terrestrial vertebrates other than birds ingesting granules of BW01 GB

	Acute risk assessment
Single application rate (worst case) [g a.s./ha]	1500
Toxicity Endpoint [mg/kg bw]	LD_{50} > 2000
Mammals ingesting granules as a source of food	
ETE [mg/kg bw]	7500
TER (Trigger)	**> 0.27 (10)**
Remark: This TER value is not considered as realistic and relevant. The calculation of the TER_A value relies on the maximum dose tested for acute toxicity, i.e. 2000 mg/kg bw. It has to be taken into consideration that no effects have been observed at this dose level and the use of a NOEL instead of an LD_{50} increases the conservatism of the risk assessment. Both ferric and phosphate ions are natural constituents of soils serving as essential nutrients in animal and plant physiology with no potential for accumulation under normal physiological conditions. Ferric pyrophosphate is also approved to be added to food sources.	
Mammals ingesting granules when eating soil-contaminated food	
DDSD [mg/kg bw]	0.15
TER (Trigger)	> 13333 **(10)**

TER values in bold indicate that risk cannot be excluded.

ETE Estimated theoretical exposure
DGritD Daily grit dose
DGD Daily granule dose
DDSD Daily dry soil dose
TER Toxicity exposure ratio

Risk of secondary poisoning is not to be expected since Ferric pyrophosphate is practically insoluble. In addition, there is no risk for mammals resulting from exposure to contaminated drinking water.

Risk Assessment

Toxicity

The mammalian toxicity endpoint for Ferric pyrophosphate that is most appropriate for acute ecological risk assessment is presented in **Table 2-1**. No toxicity was observed up to a dose of 2000 mg a.s./kg body weight, which indicates a very low toxicity of Ferric pyrophosphate. No additional acute mammalian endpoints for BW01 GB are considered relevant, due to the very low toxicity of Ferric pyrophosphate and the character of the other components of the product. For the risk assessment a LD_{50} of > 2000 mg a.s./kg bw (in fact a NOEL) is considered appropriate.

Exposure

The evaluation of the risk for mammals was performed in accordance with EFSA Journal (2009)[15]. The relevant endpoints are presented in the overview above.

[15] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

BW01 GB is a molluscicide intended to be used as slug granule up to 6 times with a 14 day interval with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in oilseed rape, with a maximum single application rate of 7 kg/ha (corresponding to 210 g a.s./ha) in winter wheat and with up to 50 kg/ha (corresponding to 1.5 kg a.s./ha) in ornamentals (worst case). For a detailed summary of the GAP uses of BW01 GB, please refer to Appendix 1, Table of intended uses. The potential exposure of mammals to BW01 GB was estimated following six applications of BW01 GB at 50 kg product/ha (worst-case).

Mammals may be exposed to Ferric pyrophosphate mainly by the consumption of contaminated food.

According to EFSA Journal (2009), mammals may be exposed to granular formulations in different ways:

a) Mammals may ingest granules as a source of food.

b) Mammals may ingest granules when they eat food contaminated with soil.

c) Mammals may consume food contaminated with residues resulting from granular applications (slug-eating mammals).

Since it can be assumed that a second to sixth application is only performed when granules of the previous application have been consumed by slugs or otherwise disappeared, no multiple application factor (MAF) was considered in the risk assessment for mammals ingesting granules of BW01 GB (exposure paths a and b). No MAF is incorporated into the risk assessment for slug-eating mammals (exposure paths c) since slugs usually feed only once at a BW01 GB granule, immediately after the application granules are available to excess and die thereafter. Therefore, it can be expected that no residues will build up and that the maximum values after the second application are not higher than after the first application.

Toxicity exposure ratios

Acute toxicity exposure ratio (TER_A)

Acute risk was calculated using the lowest acute LD_{50} value for the active substance. A TER_A value below 10 indicates a potential acute risk to mammals. The results are presented in **Table 2.1.1-1** to **Table 2.1.1-2**.

First-Tier risk assessment

a) Mammals ingesting granules of BW01 GB as a source of food

Since there is a possibility that mammals might mistake granules of BW01 GB for food, a risk assessment is conducted based on the calorific value of the granular material.

According to EFSA Journal (2009)[16] the species of concern for the first-Tier assessment is an omnivorous mammal (e.g. wood mouse with a body weight of 21.7 g). The BW01 GB granules have a caloric value of 328.75 kcal/100 g (corresponding to 13.76 kJ/g), which is comparable to cereals seeds and might substitute this food type in the diet of the indicator species.

The estimates of the food intake rate (FIR) of a certain food type in a mixed diet are based on means of daily energy expenditure of the indicator species, energy and moisture content and assimilation efficiencies of the respective food type. The FIR can be calculated as follows:

$$FIR = \frac{DEE}{FE \times \left(1 - \frac{MC}{100}\right) \times \frac{AE}{100}} \left[\text{g fresh weight/d}\right]$$

[16] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

With:		
DEE	=	Daily energy expenditure of the indicator species
FE	=	Food energy [kJ/g d.w.]
MC	=	Moisture content [%]
AE	=	Assimilation efficiency [%]

The estimated theoretical exposure (ETE), i.e. the uptake of a compound via a single food item is given by the following equation:

$$ETE = \frac{FIR}{bw} \times C \times PT \left[mg/kg\ bw/d\right]$$

With:		
FIR	=	Food intake rate of indicator species [g fresh weight/d]
bw	=	Body weight [g]
C	=	Concentration of compound in fresh diet [30000 mg a.s./kg]
PT	=	Fraction of diet obtained in treated area (number between 0 and 1)

The TER value was calculated by dividing the acute endpoint (LD_{50}) by the estimated daily exposure (ETE):

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw/day)}{ETE\ (mg/kg\ bw/day)}$$

The assessment is shown in **Table 2.1.1-1.**

Table 2.1.1-1 Acute risk assessment for mammals ingesting granules of BW01 GB as a source of food

Indicator species	**bw [g]**	**DEE [kJ]**	**Food type**	**FE [kJ/g d.w.]**	**MC [%]**	**AE [%]**	**FIR [g/d]**	**FIR/ bw**	**C [mg a.s./kg]**	**ETE [mg/ kg bw]**	**LD_{50} [mg/ kg bw]**	**TER_A (10)**
Omnivorous mammal (wood mouse)	21.7	58.83	Cereals seeds	13.76	7	84	5.47	0.25	30000	7500	> 2000	**> 0.27**

DEE Daily energy expenditure of the indicator species
FE Food energy (please refer to the confidential document on the formulation content of BW01 GB)
MC Moisture content (please refer to the confidential document on the formulation content of BW01 GB)
AE Assimilation efficiency
FIR Food intake rate of indicator species
C Concentration of compound in fresh diet
ETE Estimated theoretical exposure
TER Toxicity exposure ratio

The TER_A value for mammals ingesting granules as a source of food is below 10, indicating that a refined acute risk assessment is required.

However, the calculation of the TER_A value relies on the maximum dose tested for acute toxicity, i.e. 2000 mg a.s./kg bw. It has to be taken into consideration that no effects (no mortality, no clinical signs, no effect on the body weight gain, no pathological changes after gross examination) have been observed at this dose level and the use of a NOEL instead of an LD_{50} increases the conservatism of the risk assessment. Furthermore, testing higher doses in order to obtain a definite LD_{50} exceeding 2000 mg/kg bw, is not foreseen in the test guidelines, nor considered appropriate. As a further refinement options either an avoidance study in pens or a field study is relevant for granular formulations. Notwithstanding, due to animal welfare further vertebrate testing is not considered appropriate.

Furthermore, both components of BW01 GB - ferric and phosphate ions are natural constituents of soils serving as essential nutrients in animal and plant physiology with no potential for accumulation under normal physiological conditions. Iron is the second most abundant metal in the environment and the fourth most abundant element, building around 5.6% of the Earth's crust. The content and distribution of iron in different soil types varies but typically it is in the average range of 1 – 5% (10 – 50 g/kg). It is an essential nutrient that is required for various biochemical and physiological functions. In animal physiology it participates in electron transfer, DNA synthesis, oxygen transport and other cellular functions. Inadequate supply of this micro-nutrient results in a variety of deficiency diseases and syndromes. Also, phosphorus is an element essential for the functioning of every cell. It is a component of many important compounds such as nucleic acids, numerous coenzymes, ADP and ATP, a key compound in intracellular energy transfer. As iron, it is a natural occurring element of the terrestrial environment (appearing in concentrations of 0.01 - 0.2 %).

Bearing the above in mind, the amount of iron (III) and phosphate added by the application of BW01 GB is by several orders of magnitude smaller than the natural content commonly found in soils. Additionally, iron and, in particular, phosphate are applied in considerable amounts to agricultural soils in fertilizers.

Additionally, due to the very low solubility of Ferric pyrophosphate in water and lipids the bioavailability and absorption in the body of organisms is low (2% of an average absorption of iron from food fortified). Therefore, Ferric pyrophosphate does not possess potential for accumulation in organisms under normal physiological conditions.

Furthermore, a risk of bioaccumulation is not expected based on the function as dietary supplement in food sources. Ferric pyrophosphate has been approved as a safe source of iron added to food, even for young children and infants, processed cereal-based food and food for children, food for special medical purposes as well as total diet replacement (Regulation (EU) No 609/2013[17]). Ferric pyrophosphate is also included on the list of vitamins and mineral substances which may be used in the manufacture of food supplements according to Commission Regulation (EC) No 1170/2009[18].

Considering all the above-mentioned arguments, effects due to consumption of granules of BW01 GB can be excluded and are not further assessed.

RMS: The estimated theoretical exposure (ETE) of indicative mammal to ferric pyrophosphate ingesting granules of BW01 GB containing 2.4% of ferric pyrophosphate as a single food item (worst case scenario) was calculated according to formulas and default values given in appendix G of the Guidance document (EFSA Journal 2009; 7 (120: 1438). The ETE amounts to 7500 mg of ferric pyrophosphate/kg bw with assumption that all the energy comes from a diet composed of BW01 GB granules, which is a worst case scenario rather improbable in real life.

17 Regulation (EU) no 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. Official Journal of the European Union. Off. J. Eur. Union 29.6.2013, L181, 35-56.

18 Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. Off. J. Eur. Union 1.12.2009, L314, 36-42.

There is no data to assess a preference of mammals to use granules of BW01 GB as a food item, therefore the appropriate fraction of diet obtained in treated area (PT) cannot be set.
This estimated theoretical exposure (ETE) is much higher that a dose of 2000mg/kg bw applied in the acute toxicity limit test in rats, which did not induced mortality or clinical symptoms of acute intoxication. TER_{ac} calculated for acute single exposure using the extrapolated LD50 from a limit dose of 3228mg/kg equals to 0.43. The interpretation of TERac for acute risk assessment creates high uncertainty , because real value of LD50 of ferric pyrophosphate for mammals might be much higher than 3228mg/kg equals. For example, the LD50 for rats for ferric phosphate has been estimated to be above 5000 mg/kg bw (EFSA Journal 2015;13(1):3973).

This first-tier risk assessment points out on the unacceptable risk of acute toxic effects in mammals, but it is overconservative due to:

- lack of the acute toxic effects produced by ferric pyrophosphate observed in the acceptable acute oral toxicity study in mammals
- lack of real, oral LD_{50} for mammals which might be much higher than the extrapolated LD50
- overestimation of ferric pyrophosphate consumption with granules of BW01 GB as a source of food

The ferric pyrophosphate did not induce any symptoms of acute toxicity in mammals at the highest doses foreseen in the acceptable methods for regulatory assessment of acute toxicity. Therefore, the use of TER_{ac} for acute risk assessment posed by ferric pyrophosphate seem to be misleading and is linked with a high uncertainty since it indicate the existence of risk of acute poisoning of mammals, while a substance is known of not having acute toxicity.

b) Mammals ingesting granules of BW01 GB when eating soil-contaminated food

Mammals might be exposed to BW01 GB granules as part of ingested soil when seeking food.

The acute daily dry soil dose ($DDSD_{acute}$) is calculated according to the following equation:

$$DDSD_{acute} = 0.097 \times dosage\,[kg\ a.s./ha]$$

Dosage of BW01 GB following worst-case application in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha, is 1.50 kg a.s./ha. With the worst-case highest dosage, the dosages for the other uses in ornamentals with the application rate of 25 kg product/ha, winter wheat and oilseed rape are considered as covered.

The TER value was calculated by dividing the acute endpoint (LD_{50}) by the acute daily dry soil dose ($DDSD_{acute}$):

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw/day)}{DDSD_{acute}\ (mg/kg\ bw/day)}$$

The assessment for the worst-case use in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha is shown in **Table 2.1.1-2.** The assessment for the uses in ornamentals (Hosta, Tagetes) with the application rate of 50 kg product/ha covers also the uses in ornamentals with the application rate of 25 kg product/ha, winter wheat and oilseed rape.

Table 2.1.1-2 Acute risk assessment for mammals ingesting granules of BW01 GB when eating soil-contaminated food

Crops	Dosage [kg a.s./ha]	LD_{50} [mg/kg bw]	$DDSD_{acute}$ [mg/kg bw]	TER_A (10)
Ornamentals (Hosta, Tagetes)	1.50	> 2000	0.15	> 13333

$DDSD_{acute}$ acute daily dry soil dose

TER Toxicity exposure ratio

The TER_A value for mammals ingesting granules when eating soil-contaminated food is above 10, indicating that a refined acute risk assessment is not required.

RMS: Mammals ingesting granules when eating soil-contaminated food

The acute risk for mammals ingesting granules of BW01 GB when eating soil-contaminated food has been assessed in line with recommendations of the EU guidance document (Guidance Document on Risk Assessment for Birds & Mammals, EFSA Journal 2009; 7(12): 1438. The acute daily dry soil dose (DDSDac) for a mammal calculated according to formula and assumptions given in section 5.1.4. *Animals ingesting granules when eating soil-contaminated food* of EFSA guidance document amounted to 0.15 mg/kg bw, giving Toxicity Exposure Ration TER of > 13333 thus above a target value of 10. This first-tier assessment indicate that application of BW01 GB in accordance to GAP proposed by the applicant does not create a risk of acute toxic effects for mammals ingesting granules when eating soil-contaminated.

c) Mammals consuming other food items with residues from granular application of BW01 GB (slug-eating mammals)

Due to the use of BW01 GB as slug granules, residues in other food items but slugs are not to be expected. Ferric pyrophosphate is not a systemic substance and up-take via seedlings is not expected. Bioaccumulation and biomagnification in terrestrial food chains is not expected since Ferric pyrophosphate is virtually insoluble and there is no potential for accumulation under normal physiological conditions. However, an additional exposure scenario results from the use of BW01 GB as slug granule since mammals may consume slugs containing residues of Ferric pyrophosphate.

At present, no standardized risk assessment scheme is available for the exposure scenario of slug eating mammals. There is no data of possible contents of Ferric pyrophosphate in slugs after consumption of BW01 GB available, also there any reliable information on possible consumption of contaminated slugs by mammals.

Ferric pyrophosphate affects the calcium metabolism of slugs with the result of a disturbance of the fluid household in turn with a disruption of feeding and mucus production and such mechanism is not relevant for mammals. After consumption of BW01 GB the slugs retreat themselves and die in hidden places. Therefore, a consumption of slugs containing residues of Ferric pyrophosphate is very unlikely, as these are not achievable for mammals after consuming BW01 GB and moving to hidden places. There is also some excretion through feaces potentially lowering the content of AS in slugs.

Considering the above-mentioned arguments, effects due to consumption of slugs containing residues of Ferric pyrophosphate can be excluded and are not further assessed.

RMS. As stated in the EU Guidance document At present, no standardised schemes are available for assessing the risk of residues of granular formulations in other food items such as earthworms and plant seedlings. This is mainly due to the lack of transfer factors for calculating concentrations in the food items for birds and mammals, e.g. transferring the load of granules to a concentration in the earthworm and the seedling. The probability of consumption by of other food items with residues from granular application of BW01 GB is negligible. The justification provided by the applicant is acceptable.

Short-term toxicity exposure ratio (TER_{ST})

Since Ferric pyrophosphate is considered to be not toxic to mammals and furthermore does not have potential for accumulation, an addition of toxic effects cannot be expected. According to EFSA Journal (2009)[19], no assessment is required.

[19] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Long-term toxicity exposure ratio (TER_{LT})

BW01 GB can be regarded as safe for mammals due to the natural occurrence of its both components - ferric and phosphate ions in the environment, the occurrence of Ferric pyrophosphate as the dietary supplementation in food sources and the general toxicological properties e.g. Ferric pyrophosphate has no potential for accumulation under normal physiological conditions.

It must also be considered that there had been no acute effects at all at the highest dose tested (2000 mg a.s./kg bw) in rat. Also, the 90-days repeated dose study conducted with rats excluded sub-chronic effects of the active substance and chronic toxicity up to the highest tested concentration of 1000 mg/kg b.w.

As a matter of fact, BW01 GB is applied when slug infestation occurs. Only when the applied product is eaten up by slugs, a second application is recommended. Thus, continuous long-term exposure of mammals to the product is not expected.

Effects of secondary poisoning

According to the EC Guidance Document on Risk Assessment for Birds and Mammals[20], substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains. Bioaccumulation of Ferric pyrophosphate is not expected since the Ferric pyrophosphate is practically insoluble and does not possess potential for accumulation under normal physiological conditions. Therefore, a risk assessment for secondary poisoning is not required for Ferric pyrophosphate.

Background levels of iron and phosphate in soil , use as a food supplement, studies showing a low risk to birds and mammals

Natural amount of iron and phosphate occurring in soil is 10 000 – 50 000 mg/kg and 275 – 16 500 mg/kg, respectively (DAR Vol. 3 CP, B.8.1).
Ferric pyrophosphate is generally recognized as safe by USA Food and Drug Administration, which is presented in 'Animal drugs, feeds, and related products' in Code of Federal Regulations Title 21 (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=582&showFR=1&subpartNode=21:6.0.1.1.25.6 access: 13.05.19) and is permitted to be added to animal feeds as nutritional dietary supplement.
(https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=582&showFR=1&subpartNode=21:6.0.1.1.25.1 access: 13.05.19).
Ferric pyrophosphate has been approved as a safe source of iron added to food, even for young children and infants, processed cereal-based food and food for children, food for special medical purposes as well as total diet replacement (Regulation (EU) No 609/2013 [21]). Ferric pyrophosphate is also included on the list of vitamins and mineral substances which may be used in the manufacture of food supplements according to Commission Regulation (EC) No 1170/2009 [22].

RMS: Overall conclusion regarding risks to birds and mammals:

Taking into account presented data on toxicity of active substance, being an inorganic substance, and data on toxicity of product BW01 GB as well as information on ubiquitous occurrence of its main structural components: iron and inorganic phosphorus compounds in biological organisms and in soil, no unacceptable acute and long-term risk to birds or other terrestrial vertebrates is anticipated when formulation BW01 GB is applied as molluscicide in a form of granules at doses 0.12 - 1.5 kg of the active substance per hectare from 1 to 6 times per year. As indicated in the evaluation of the fate and behaviour in the environment the application of product BW01 GB according to GAP will have negligible effect on increase of actual content of these elements on the environment due to the migration of iron naturally present in soil that results from the weathering of mineral material or phosphate related ions that are applied to agricultural soils as fertiliser. The concentration of Ferric pyrophosphate

[20] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

in BW01 GB granules equals 30 000mg/kg, which is in the same order of magnitude as concentration of iron and phosphate in soil since the natural amount of iron and phosphate occurring in soil is 10 000 – 50 000 mg/kg and 275 – 16 500 mg/kg, respectively (DAR Vol. 3 CP, B.8.1). It is noted that in the acceptable acute and long-term/reproduction toxicity studies Ferric pyrophosphate or a product BW01 GB did not produce any toxic effects in birds and in mammals, which would justify recognition of this substance as harmful or justify the classification of this substance to any health or environmental hazard classes according to criteria of Regulation (EC) No 1272/2008. The quantitative acute and long term risk assessment for birds and mammals is considered overconservative since it was not possible to determine the endpoints used for risk calculation such as LD_{50} or NOEC due to very low toxicity of the substance for birds and mammals. The estimated/extrapolated doses used for risk assessment were much lower than the real values of these endpoints if they exist at all. The higher doses or concentration of the test substance which could allow determination of these endpoints were not used in the studies since they would be much higher than the limit dose or concentration allowed by the test guidelines for these methods. Still some approaches to quantitative risk assessment demonstrated low, acceptable acute risk for birds ingesting granules of BW01 GB when seeking seeds as food, for birds ingesting granules of BW01 GB when eating soil-contaminated and for mammals ingesting granules when eating soil-contaminated food. Summing up it is concluded that application of a product BW01 GB in line with the proposed in GAP used pattern will not create an unacceptable risk for birds and mammals.

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B.9.3 Effects on Aquatic Organisms

B.9.3.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

B.9.3.1.1 Fish

B.9.3.1.1/01 Fish acute toxicity

Report: [redacted]2014, Fish Acute Toxicity Test according to OECD Guideline No 203

Study code: 0001/0091/E

Guideline: OECD Guideline 203

GLP: yes

Executive Summary:

The acute toxicity study of the test item, granules BW01 GB for rainbow trout (*Oncorhynchus mykiss*) was conducted. The aim of the study was to determine LC_{50}, LC_0 and LC_{100} values calculated on the basis of observed fish mortality symptoms after 24, 48, 72 and 96 hours of exposure. The test material is non toxic in the concentration 7,4 mg/l (nominal concentration 100 mg/l), in suspension, being the maximum concentration used in the fish toxicity test. During the experiment, neither mortality of fish was observed, nor signs of intoxication in tested concentration.

A. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BW01 GB

Description: turquoise granules

Content of active substance: 3% of iron pyrophosphate

Batch: 032014-P82

Storage: cool and dry place

2. Vehicle and/or positive control: tap water: carbonate hardness 195mg/l / potassium dichromate

3. Test animals

Species: rainbow trout (*Oncorhynchus mykiss*)

Age: juveniles

Size: 5,2 – 5,6 cm (preliminary test); 5,1 – 5,8 cm (definitive test)

Weight: 1,11g – 1,24g (preliminary test); 1,05g – 1,27g (definitive test)

Source: [redacted]

Acclimation period: 17 days

Diet: standard granulated fish food produced by ALLER AQUA

Environmental conditions

Temperature: 14,9°C - 15,3 °C

Photoperiod: fluorescent lighting in a daily cycle - 16 h day and 8 h night

Concentration of the oxygen: not lower than 60%.

Total hardness: 3,9 ± 0,3 mval/l

Conductivity: 561 µS/dm^3

B. STUDY DESIGN AND METHODS:

1. In life dates: 14.04.2014 - 18.04.2014

2. Experimental treatments

Preliminary test

In the preliminary test 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, 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 semi-static system (solutions were exchanged at 24 hours) and lasted for four days.

Definitive test

The test was prepared in a single replicate with seven fish introduced into each aquarium (maximum 1 gram of fish per 1 liter of medium). 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 by a fresh one at 24 hours. Because no mortality was observed in a preliminary test, a limit test was applied with only single concentration - 100 mg/l being the maximal concentration using in the fish toxicity test.

Reference test

Experiment with reference substance - potassium dichromate ($K_2Cr_2O_7$) was performed. The aim of test was checking the physiological condition of fish. Concentration which caused mortality of 50% of tested fish population (LC_{50} value) after 24, 48, 72 and 96 hours of exposure as well as LC_0 and LC_{100} values were determined. 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.

3. Observations

Observations of fish mortality and signs of intoxication of the test material were carried out in each experimental aquarium at 3, 6, 24, 48, 72 and 96 h after the start of the experiment. In none of the tested concentrations fish mortality or signs of intoxication like loss of balance, change in swimming, breathing and pigmentation were observed.

4. Statistical calculations

LD_{50}, LD_{20} and LD_{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 correct

5. Deviations to the Guideline

There were no deviations from the Guideline.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.3-1 Final calculated values*

	24 h	48 h	72 h	96 h
LC 50 [mg/l]	n/a**	n/a**	n/a**	n/a**
LC 0 [mg/l]	n/a**	n/a**	n/a**	n/a**
LC 100 [mg/l]	n/a**	n/a**	n/a**	n/a**
NOEC [mg/l]	≥ 7,4	≥ 7,4	≥ 7,4	≥ 7,4
LOEC [mg/l]	> 7,4	> 7,4	> 7,4	> 7,4

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

Table 9.3-2 Acute toxicity of BW01 GB to rainbow trout

BW01 GB	Time point [h]	Abnormalities/ Sublethal Effects	Mortality	Total mortality
Preliminary test				
Control	3 6 24 48 72 96	Not observed	0 0 0 0 0 0	0
0,1	3 6 24 48 72 96	Not observed	0 0 0 0 0 0	0
1	3 6 24 48 72 96	Not observed	0 0 0 0 0 0	0
10	3 6 24 48 72 96	Not observed	0 0 0 0 0 0	0
100	3 6 24 48 72 96	Not observed	0 0 0 0 0 0	0
Definitive test				
Control	3 6 24 48 72 96	Not observed	0 0 0 0 0 0	0
100	3 6 24 48 72 96	Not observed	0 0 0 0 0 0	0

B. OBSERVATIONS

During the experiment, neither mortality of fish was observed, nor signs of intoxication in tested concentration.

III. CONCLUSIONS

The test material is non-toxic in the concentration 7,4 mg/l (nominal concentration 100 mg/l), in suspension, being the maximum concentration used in the fish toxicity test. During the experiment, neither mortality of fish was observed, nor signs of intoxication in tested concentration.

RMS comments:

The acute toxicity study of BW01 GB for on fish performed in GLP conditions and according OECD TG 203 is considered valid and established endpoints may be used for risk assessment. 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 nominal concentration 100 mg of iron pyrophosphate in 1 L litre of medium. Based on iron measurements in medium and in the product it was calculated that the concentration of BW01 GB in aqueous medium for fish amounted to 7.4 mg/l.

The study is considered acceptable with following endpoints:

96h LC_{50} for fish is greater than 7.4 mg/l (nominal concentration 100 mg /L)

Since no signs of intoxication of fish were observed during 96h test a NOEC (no observed effect concentration) of BW01 GB in these test conditions is ≥ 7.4 mg/l .

B.9.3.1.2 Aquatic invertebrates

B.9.3.1.2/01 Daphnia sp. Acute Immobilization Test

Report: Winkler J. 2014, Daphnia sp. Acute Immobilization Test according to OECD Guideline No 202

Study code: 0001/0090/E

Guideline: OECD 202

GLP: yes

Executive Summary:

The acute *Daphnia magna* immobilization test for test item, BW01 GB was conducted. The aim of the study was to determine EC_x, NOEC and LOEC values calculated for the particular observed effects. During the experiment and performed statistical calculations it was shown, that the tested item BW01 GB used in its nominal concentration as the suspension, and being the maximal used concentration according to the OECD 202 Guideline - 100mg/l and in actual (measured) mean one 7,055 mg/l, has no effect on the immobilization of Daphnia magna after 48 hours of experience, and therefore does not exhibit ecotoxic properties. Also, in none repetition of test item concentration intoxication effects were observed. According to OECD 202 Guideline, if there is an evidence that the concentration measured at the end of the test is with ± 20% of initial measured concentration, statistical calculations may be performed on the basis of nominal concentrations. During the chemical analysis, the actual concentrations of BW01 GB in the solutions were determined: 7,12 mg/l at the beginning of the experiment and 6,99 mg/l at the end of the experiment. Due to the fact that mentioned stability criteria were met, basing on the obtained results the calculations were performed for nominal concentrations. Based on the results, the calculation

of EC_x, NOEC, LOEC was impossible due to mathematical reasons. Limit test performed at maximal test item concentration 100 mg/l (mean measured – actual one 7,055 mg/l) demonstrated that the EC_{50} is greater than this concentration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: BW01 GB

Description: turquoise granulate

Content of active substance: 3% of iron pyrophosphate

Batch: 032014-P82

Storage: cool and dry place

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

3. Test animals

Species: *Daphnia magna*

Age: no older than 24h

Source: own breeding of research laboratory

Diet: green algae, suspension of lyophilized cyanobacterias spirulina,yeast

Acclimation period: without acclimation

4. Environmental conditions

Temperature: 20 ± 2°C

Photoperiod: 400 - 1000 lux in a daily cycle - 16 h day and 8 h night

Oxygen concentration: more than 3,0 g/l

pH: 7,31- 7,93

Carbonate hardness: approx. 35,72 mg $CaCO_3$/l

B. STUDY DESIGN AND METHODS:

1. In life dates: 31.03.2014 to 04.04.2014

2. Experimental treatments

Preliminary test

In the preliminary test control and the following nominal concentrations of test item were used: 1 mg/l, 10 mg/l, 100 mg/l. Each concentration was prepared in four replicates, with 5 organisms introduced into each replicate. The feeding of daphnia was suspended 24 hours before the beginning of the experiment. As test vessels 100 ml clean, glass beakers were used, 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.

Definitive test

On the basis of preliminary test in the definitive test the single, maximal concentration of the test item (nominal concentration 100mg/l) and control were used. The study was conducted as the limit test. Both the concentration of the test item and the control were tested in four replicates of five daphnia introduced into each replicate. The study was in static system - the concentrations of the test item were not replaced during the experiment by the fresh ones. Freshly prepared solutions of concentrations of the test item and old solutions at the end of the study were passed for the chemical analysis.

The reference test

The acute *Daphnia magna* immobilization test for reference test item, potassium dichromate was conducted. The aim of the study was to determine EC_x value for particular observed. The reference test item concentration was tested using following concentrations: 0,1mg/l; 0,2mg/l; 0,4mg/l; 0,8mg/l; 1,6mg/l. The concentrations were prepared by directly dissolving the test material in the ISO medium and preparing stock solution. Each concentration was prepared by dilution the stock solution. Each concentration was thoroughly mixed before use. Each reference test item concentration as well as control were prepared in four replicates, with 5 organisms introduced into each replicate. The study was performed in static system – during the experiment the reference test item solutions were not replaced for the freshly prepared.

3. Observations

During the experiment, the temperature of the water (additional test vessel arranged directly next to the test vessels) was measured continuously at intervals of 1 hour. At the beginning and at the end of the test pH and dissolved oxygen values were measured in the control and all tested concentrations. The measurements were performed in single replicate of each concentration. At the beginning and end of the test, the light intensity in the test room was measured. Observations of the number of immobilized daphnia in each test vessels as well as changes in the appearance were conducted after 24 and 48 hours from the beginning of the experiment.

4. Deviations from the Guideline

According to the OECD Guideline 202 medium carbonate hardness should be between 140 - 250 mg/l of $CaCO_3$. In the current experiment, the measured medium carbonate hardness was 2ºn which is approx. 35,72 mg $CaCO_3$ per liter (1ºn = 17,86 $CaCO_3$ mg/l). The medium was prepared with deionized water. During the process of ionization inter alia the carbon salts are removed from water which are responsible for calcium carbonate hardness of water. In the current experiment, there was no mortality or signs of intoxication in any of the tested item concentrations nor control observed. Therefore it is considered that the deviation did not affect the experiment neither the obtained final results.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.3-3 Summary of results for all endpoints

0 – 48 h			
EC_{10} [mg/l]	n/d	95%-CL upper	n/ d
		95%-CL upper	n/d
EC_{20} [mg/l]	n/d	95%-CL upper	n/d
		95%-CL upper	n/d
EC_{50} [mg/l]	n/d	95%-CL upper	n/d

		95%-CL upper	n/d
NOEC [mg/l]	n/d		
LOEC [mg/l]	n/d		

* n/d - not determined due to mathematical reasons or inappropriate data

B. OBSERVATIONS

During the study immobilization of daphnia as well as intoxication symptoms did not occur in any replication of the test item concentration nor control.

III. CONCLUSIONS

The current experiment indicated, that test item BW01 GB, has no effect on the immobilization of after 48 hours of experience, and therefore does not exhibit ecotoxic properties against *Daphnia magna* in nominal concentration 100mg/l and in actual (measured) mean one - 7,055 mg/l. Based on the results, the calculation of EC_x, NOEC, LOEC was impossible due to mathematical reasons. Limit test performed at maximal test item concentration 100 mg/l (mean measured – actual one 7,055 mg/l) demonstrated that the EC_{50} is greater than this concentration.

RMS comments:

The acute immobilization test on *Daphnia magna* by BW01 GB performed in GLP conditions and according OECD TG 202 is considered valid and established endpoint may be used for risk assessment.

One deviation from the test guideline was noted, but it is considered that it did not have an impact on the study results.

The limit test was performed with the suspension of BW01 GB in water containing 100 mg of BW01 GB in 1 litre of medium and with actual mean concentration (calculated based on measurements of iron in aqueous medium and in product) equal 7,055 mg/l.

The study is considered acceptable with following endpoints

96h EC_{50} is greater than 7,055 mg BW01 GB /L, and greater than 100mg BW01 GB /L as nominal concentration.

B.9.3.1.3 Algae

Acute toxicity studies for fish and aquatic invertebrates both for the active substance (see B.9.2.1 and B. 9.2.4.1) and the formulation (see B.9.3.1.1 and B.9.3.1.2) indicate that the formulation is not more toxic than the active substance. Thus, the data on the active substance might be treated as the worst-case scenario and based on this data (see B.9.2.6) it might be predicted that the BW01 GB plant protection product will not have an effect on algae growth.

B.9.3.1.4 Aquatic macrophytes

Studies on effects of VITROL GB (BW01 GB) on aquatic macrophytes were not required since Ferric pyrophosphate is a molluscicide and does not exhibit herbicidal activity.

B.9.3.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

B.9.3.2.1 The study of the long-term and chronic toxicity to fish

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

Study code: 0001/0109/E

Guideline: OECD Guideline 210

GLP: yes

Executive Summary:

The long-term and chronic toxicity study of the test item, BW01 GB for early-life stage of Zebrafish (*Danio rerio*) was conducted. The aim of the study was to determine the highest observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). Based on the research and statistical calculations indicated that the test material, BW01 GB, at the concentration 4,6 mg/l (nominal concentration - 10 mg/l), has no effect on the percentage hatching, the survival or growth of organisms (expressed as weight and length change). Based on the obtained results the NOEC value for each experience points was determined - in each case obtained NOEC ≥ 4,6 mg/l. The test material is non-toxic for early-life stage of Zebrafish (*Danio rerio*).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BW01 GB

Description: turquoise granules

Content of active substance: 3% of iron pyrophosphate

Batch: 032014-P82

Storage: cool and dry place

2. Vehicle and/or positive control: tap water

3. Test animals

Species: Zebrafish (*Danio rerio)*

Age: n/a

Weight: n/a

Source: [redacted]

Diet: standard aquarium fish food (Tropical) and freshly hatched individuals of *Arthemia salina*, ad libitum

Acclimation period: 28 days

4. Environmental conditions

Temperature: 26,20°C - 27,50°C

Photoperiod: fluorescent lighting in a daily cycle, 16 h day: 8 h night

Air change: continuously aerated

Carbonate hardness: 150-250mg/l

B. STUDY DESIGN AND METHODS:

1. In life dates: 18th August - 19th September 2014

2. Experimental treatments

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 experience. The experiment was carried out in a semi-static system (solutions were exchanged at 24 hours) and lasted for four days.

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 - 10 mg/l was used. 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. The test lasted 30 days from the hatching. The experiment was carried out in a semi-static system. Solutions were exchanged by a fresh one at 72 hours.

3. Observations

Observations of fish mortality and signs of intoxication of the test material were carried out in each experimental aquarium at 3, 6, 24, 48, 72 and 96 h after the start of the experiment.

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. Moreover 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.

4. Statistical calculations

Data analysis was performed using statistical analysis software ToxRat Professional. Calculations were made on the basis of the geometric mean of the measured content of the test material (80 µg iron/l), because during the experiment the content of the test material was not maintained at the level of 80 - 120% of the nominal value.

5. Deviations to the Guideline

There were no deviations from the Guideline.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.3-4 Fish mortality in the preliminary test – raw data

	3 h	6 h	24 h	48 h	72 h	96 h
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 9.3-5 Results of the definitive test – control

Day	**Number of hatched larvae**				**Number of dead eggs (J), embryos (E), larvae (L), fish (R)**				**Intoxication symoptoms**			
	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 symptoms	no symptoms	no symptoms	no symptoms
2	14	14	14	14	6 (J)	6 (J)	6 (J)	6 (J)	no symptoms	no symptoms	no symptoms	no symptoms
3	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
4	-	-	-	-	0	0	1 (L)	0	no symptoms	no symptoms	no symptoms	no symptoms
5	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
6	-	-	-	-	1 (R)	1 (R)	0	0	no symptoms	no symptoms	no symptoms	no symptoms
7	-	-	-	-	0	0	1 (R)	0	no symptoms	no symptoms	no symptoms	no symptoms
8	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
9	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
10	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
11	-	-	-	-	1 (R)	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
12	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
13	-	-	-	-	0	1 (R)	0	0	no symptoms	no symptoms	no symptoms	no symptoms
14	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
15	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
16	-	-	-	-	0	0	1 (R)	0	no symptoms	no symptoms	no symptoms	no symptoms
17	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
18	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
19	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
20	-	-	-	-	0	1 (R)	0	0	no symptoms	no symptoms	no symptoms	no symptoms

21	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
22	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
23	-	-	-	-	1 (R)	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
24	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
25	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
26	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
27	-	-	-	-	0	0	1 (R)	0	no symptoms	no symptoms	no symptoms	no symptoms
28	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
29	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
30	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
31	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
32	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms

1-3 day larvae stadium 5-32 day fry stage

Table 9.3-6 Results of the definitive test – tested concentration (10 mg/l)

Day	**Number of hatched larvae**				**Number of dead eggs (J), embryos (E), larvae (L), fish (R)**				**Intoxication symoptoms**			
	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 symptoms	no symptoms	no symptoms	no symptoms
2	14	13	14	14	6 (J)	7 (J)	6 (J)	6 (J)	no symptoms	no symptoms	no symptoms	no symptoms
3	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
4	-	-	-	-	0	0	1 (L)	0	no symptoms	no symptoms	no symptoms	no symptoms
5	-	-	-	-	1 (R)	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
6	-	-	-	-	1 (R)	0	1 (R)	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
7	-	-	-	-	0	1 (R)	0	0	no symptoms	no symptoms	no symptoms	no symptoms
8	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
9	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
10	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
11	-	-	-	-	0	0	1 (R)	0	no symptoms	no symptoms	no symptoms	no symptoms
12	-	-	-	-	1 (R)	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
13	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
14	-	-	-	-	0	1 (R)	0	0	no symptoms	no symptoms	no symptoms	no symptoms
15	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
16	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms

17	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
18	-	-	-	-	0	1 (R)	0	0	no symptoms	no symptoms	no symptoms	no symptoms
19	-	-	-	-	0	0	1 (R)	0	no symptoms	no symptoms	no symptoms	no symptoms
20	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
21	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
22	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
23	-	-	-	-	1 (R)	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
24	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
25	-	-	-	-	0	1 (R)	0	0	no symptoms	no symptoms	no symptoms	no symptoms
26	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
27	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
28	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
29	-	-	-	-	0	0	0	1 (R)	no symptoms	no symptoms	no symptoms	no symptoms
30	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
31	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms
32	-	-	-	-	0	0	0	0	no symptoms	no symptoms	no symptoms	no symptoms

1-3 day larvae stadium 5-32 day fry stage

Table 9.3-7 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 [mg]	**Length [mm]**	**Weight [mg]**	**Length [mm]**	**Weight [mg]**	**Length [mm]**	**Weight [mg]**	**Length [mm]**
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

B. OBSERVATIONS

In the preliminary test none of the tested concentrations fish mortality or signs of intoxication like loss of balance, change in swimming, breathing and pigmentation were observed. Dead fish were not checked for the presence of particles of the test material in the gills, because based on the research it was demonstrated that the test material has no affect on the survival of the organisms.

III. CONCLUSIONS

Based on the research and statistical calculations indicated that the test material, BW01 GB, at the concentration 4,6 mg/l (nominal concentration - 10 mg/l), has no effect on the percentage hatching, the survival or growth of organisms (expressed as weight and length change). Based on the obtained results the NOEC value for each experience points was determined - in each case obtained NOEC ≥ 4,6 mg/l. The test material is non-toxic for early-life stage of Zebrafish (*Danio rerio*).

RMS comments:

The long-term and chronic toxicity study of BW01 GB to fish (early-life stage of Zebrafish (*Danio rerio*) performed in GLP conditions and according OECD TG 210 is considered valid. No significant deviations from the test guideline were noted. All validity criteria were met. The definitive test was performed with one nominal concentration 10 mg of the test item in 1 L litre of aqueous medium. Based on iron measurements in medium and in the product it was calculated that the concentration of BW01 GB in aqueous medium for fish amounted to 4.6 mg/l.

The study is considered acceptable with following endpoint:

30-day NOEC in early-life stage of Zebrafish test is greater than or equal to nominal concentration 10 mg product/L (≥ 4.6 mg product/L - measured concentration).

ED properties

The results of the study can also be used to evaluation of endocrine disruption (ED) properties. 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.

According to the ED criteria[21], 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 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.

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 therefore the results of the test indicate that ferric pyrophosphate should not considered as having endocrine disrupting properties for fish.

B.9.3.2.2 The study of reproduction in Daphnia

Report: Winkler J. 2014**,** Daphnia reproduction test according to OECD 211 Guideline

21 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

Study code: 0001/0111/E

Guideline: OECD 211

GLP: yes

Executive Summary:

The reproduction test for Daphnia (*Daphnia magna* Straus*)* for test item BW01 GB, was conducted. The main aim of the study was to determine the influence of the test item on Daphnia's reproduction and growth. In addition the adults' mortality was evaluated as well was the observation of the other negative effect of test item were conducted, like loss of the reproduction abilities. Finally the detailed information in the fields of observed effects were obtained. The final points of the experiment were EC_x, NOEC and LOEC values.

Table 9. 3-8 Definitive test endpoints values

Parameter	EC_{10}	EC_{20}	EC_{50}	NOEC	LOEC
Cumulative offspring number per introduced parent (21 days)	215,895 (n/d*)**	225,625 (n/d*)**	245,477 (n/d*)**	100,00	250,000
Cumulative offspring number per survival parent (21 days)	n/d*	n/d*	n/d*	≥ 250,000	> 250,000
Cumulative immobilized offspring number (21 days)	n/d*	n/d*	n/d*	n/d*	n/d*
Cumulative number of abandonment eggs (21 days)	n/d*	n/d*	n/d*	n/d*	n/d*
Cumulative number of male offspring (21 days)	n/d*	n/d*	n/d*	n/d*	n/d*
Mortality (21 days)****	1,435 (n/d*)**	7,263 (n/d*)**	161,591 (n/d*)**	≥ 250,000	> 250,000
Length of parental specimens	249,779 (16,925 – 3895,033)**	251,093 (0,000 – 733301952,000)**	253,624 (0,000 – 3,17)**	100,00*****	250,00*****
Time of production of the first offspring	n/d*	n/d*	n/d*	n/d*	n/d*

* n/d – not determined due to mathematical reasons of inappropriate data
** lower – upper 95%-CI
***inf – infinity
**** no statistical significance in relation to the control
*****values determined basing on the actual observations, not mathematically

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BW01 GB

Description: turquoise granulate

Content of active substance: 3% of iron pyrophosphate

Batch: 032014-P82

Storage: Dry and cool place

2. Vehicle: ISO

3. Test animals

Species: *Daphnia magna*

Age: no older than 24 h

Source: own breeding of research laboratory

Diet: suspension of *Pseudokirchneriella subcapitata* algae, 3 times a week

Acclimation period: n/a

4. Environmental conditions

Temperature: 20°C ± 2 °C

Photoperiod: 1000 – 1500 luks, 16 h of light : 8 h of darkness

pH: 6 - 9

Oxygen concentration: not less than 3 mg/l

Total hardness: 140 mg/l ($CaCO_3$)

B. STUDY DESIGN AND METHODS:

1. In life dates: 30.07 to 20.08.2014 and 22.08 to 12.09.2014

2. Experimental treatments

Preliminary test

For each concentration (1 mg/l; 10 mg/l; 100 mg/l; separation factor 10) and (0 mg/l) two replicates were used with a single daphnia introduced into each replicate. Test lasted for 21 days. In the study the test item impact on adult daphnias mortality was conducted. In addition, the offspring number was recorded. During the study the solutions were not sampled and weren't passed to the analysis.

Definitive test

During the definitive test, control (0 mg/l) and the following test item concentrations were used: 6,4 mg/l; 16 mg/l; 40 mg/l; 100 mg/l and 250 mg/l (separation factor 2,5). Each test item concentration as well as control consisted of ten replicates. Test was conducted in semi-static system with solutions renewal every 48-72 hours (on the basis of the stability test results). Freshly prepared and old solutions of test item concentrations were passed to the chemical analysis.

3. Observations

During the experiment the following observations were performed:

- the number of offspring in each test vessel each day of the experiment (young were removed at once, during the observation), the number of dead offspring and abandoned eggs, as well as time of the production of the first offspring were recorder;

- mortality of the parent animals every day of the test - caused by the test item (no cases of unintentional killing occurred);
- length of the adult animals at the end of the experiment;
- coefficient of variation (% CV) of offspring numbers in the control vessels at the end of the experiment – it did not exceed 25 % (it was 16,2%),
- appearance of the tested solutions – freshly prepared and old ones;
- also untypical behavior of daphnias and intoxication symptoms were recorded.

During the experiment the following measurements were performed: oxygen concentration [mg/l], and the pH value of the medium, hardness, temperature of the medium, light intensity, analytical measurements.

4. Statistical calculations

Obtained results were statistically analyzed in ToxRat Pro Professional.

5. Deviations from the Guideline/Study Plan

During the preliminary test, external probe of temperature recorder has been ejected. Therefore, part of the data of temperature readings (Water) from an additional vessel with deionized water, positioned directly by the test vessels are missing. In addition, there is a possibility that recorded measurements may be incorrect due to improperly connected probe. Accordingly, in the present experiment the measurements results of internal probe (Air) were taken into consideration. It is assumed that the air environment temperature and deionized water from the additional vessel temperature were similar, thus the deviation did not affect the study performance and the final results.

On the first day of the experiment recorded temperature (1 measurement) exceeded the upper limit (22ºC) for 1,3ºC. This deviation was the only one and most probably resulted from the heating device before the experiment, which was configured in a different room of a higher environment temperature. Following measurement, made 1 hour later was in the accordance range of OECD 211 Guideline, thus the deviation did not affect the study performance and the final results.

During the definitive study, due to incorrect recorder configuration, the temperature was measured from 21st August to 11th September 2014, thus reading results from the last definitive test day are missing (12.09.2014). Whereas, the temperature was measured directly in the beakers, in a single replicates of the control and the highest test item concentration. The measured temperatures were within the acceptable range, thus the deviation did not affect the study performance and the final results.

In the current experiment the calculations were performed for the nominal concentrations of test item, despite the lack of stability of the test item solutions ± 20%. The lack of stability occurred in a single analysis from the minimal solution sampled on 1st September 2014. The stability was demonstrated for all other solutions, thus it was assumed, that this deviation (concentration of test item higher in old solution than in freshly prepared) is connected with analysis methodic – determination of iron ions, which may origin not only from the distribution of the test item but also from the metabolic products of organisms – daphnias and feeder algae. In addition test item was tested as the suspension prepared by directly placing test item into the solution. Thus, concentration in the filtered solution is not identical with the nominal. Accordingly, this deviation was assumed irrelevant and calculations were performed for the nominal concentrations, which did not affect the study performance and the final results.

Test item – BW01 GB demonstrated stability in test conditions in the higher tested concentration 100 mg/l. In the lower concentration 0,1 mg/l lack of stability was demonstrated. Due to the characteristics of the test item (poorly

soluble in water) and analysis methodic (determination of iron ions in solution), it was assumed, that the results for the lower concentration 1mg/l maybe excluded from the evaluation. In addition, the results for filtrated suspensions of samples do not correspond to the actual content of test item in the solution – suspension of formulation in the medium. Test item is not a volatile thus it cannot wane from the system. In connection with above, preliminary and definitive tests were conducted in semi-static system with medium renewal every 48-72h. According to OECD 211 in the case, where there is an evidence that test item concentrations in the old solutions during their renewal are within ±20% of the concentration of the fresh solutions of test item, statistical calculations may be performed for the nominal concentrations. In the current study in the stability test it was demonstrated that except two analyses. Accordingly, due to demonstrated stability for all other solutions, it is assumed that this deviation is insignificant and the calculations and the results were elaborated on the basis of nominal concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.3-9 Produced offspring number – preliminary test

Concentration [mg/l]	Replicate	Sum	Sum of replicates
Control	Beaker 1	62	130
	Beaker 2	68	
1	Beaker 1	83	173
	Beaker 2	90	
10	Beaker 1	59	141
	Beaker 2	82	
100	Beaker 1	120	136
	Beaker 2	16	

Table 9.3-10 Body length of surviving adult daphnias – preliminary test

	Concentration [mg/l]			
Replicate	**Control**	**1**	**10**	**100**
1 [mm]	3,61	3,58	3,67	3,7
2 [mm]	3,69	3,64	3,68	2,6
Number of replicates	2	2	2	2
Mean [mm]	3,65	3,61	3,68	3,15
Standard deviation	0,06	0,04	0,01	0,78
Coefficient variation %	1,5	1,2	0,2	24,7

Table 9.3-11 Preliminary test endpoints values

Parameter	EC_{10}	EC_{20}	EC_{50}	LOEC	NOEC
Cumulative offspring number per introduced parent (21 days)	113,7 (0,000 – 77568456,0)*	386,2 (n/d)**	n/d**	>100,000	≥100,000
Cumulative offspring number per surviving parent (21 days)	113,7 (0,000 – 77568456,0)*	386,2 (n/d)**	n/d**	>100,000	≥100,000

*upper – lower 95% - Cl
**n/d – not determined due to the mathematical reasons or inappropriate data or no effect

Table 9.3-12 Final results for observed effects, preliminary test

Concentration [mg/l]	Mean offspring number	Comulative number of produced offspring per introduced parent [% reduction]	Cumulative number of produced offspring per surviving parent [% reduction]
Control	65	-	-
1	86,5	-33,1	-33,1
10	70,5	-8,5	-8,5
100	68	-4,6	-4,6

Table 9.3-13 The final results for the observed effect - the total number of produced offspring per introduced parent, definitive test

Concentration [mg/l]	Cumulative offspring number produced per introduced parent animal	Mean offspring number produced per introduced parent animal	% Reduction	Statistical significance
Control	684	68,4	n/d	n/a
6,4	694	69,4	-1,5	-
16	702	70,2	-2,6	-
40	783	78,3	-14,5	-
100	948	94,8	-38,6	-
250	326	32,6	52,3	+

+ significant; - not significant; *not possible to determine; n/a not applicable

Table 9.3-14 The final results for the observed effect – cumulative number of produced offspring per surviving parent, definitive test

Concentration [mg/l]	Cumulative offspring number produced per surviving parent after 21 days of exposure	Mean offspring number produced per surviving parent after 21 days of exposure	% Reduction	Statistical significance
Control	520	65,0	n/d	n/d
6,4	616	77,1	-18,7	-
16	567	81,0	-24,6	-
40	584	97,3	-49,7	-
100	838	104,8	-61,2	-
250	100	33,3	48,7	-

+ significant; - not significant; *not possible to determine; n/d not applicable

Table 9.3-15 Mortality of adult daphnias, definitive test

Concentration [mg/l]	Introduced	Surviving at the end of the experiment	% Mortality	Statistical significance
Control	10	8	20	n/d
6,4	10	8	20	-
16	10	7	30	-
40	10	6	40	-
100	10	8	20	-
250	10	3	70	-

+ significant; - not significant; *not possible to determine; n/d not applicable

Table 9.3-16 Mortality of adult daphnias, definitive test

Concentration [mg/l]	Introduced	Replicates with dead Daphnias	Day of death	Number of the survivors at the end of the experiment	% Mortality	Statistical significance
Control	10	7	20	8	20	n/d
		10	20			
6,4	10	8	15	8	20	-
		9	15			
16	10	1	17	7	30	-
		8	16			
		10	16			
40	10	1	17	6	40	-
		7	16			
		8	17			
		10	17			
100	10	1	10	8	20	-
		7	21			
250	10	2	15	3	70	-
		3	15			
		4	15			
		5	19			
		7	15			
		8	18			
		10	14			

+ significant; - not significant; *not possible to determine; n/d not applicable

Table 9.3-17 Body length of adult surviving daphnias– definitive test

Replicate	Concentration [mg/l]					
	Control	**6,4**	**16**	**40**	**100**	**250**
1 [mm]	3,67	3,69	-	-	-	3,66
2 [mm]	3,71	3,65	3,63	3,92	3,64	-
3 [mm]	3,58	3,74	3,71	3,98	3,98	-
4 [mm]	3,7	3,64	3,7	3,7	3,79	-
5 [mm]	3,59	3,71	4,05	3,89	3,99	-
6 [mm]	3,6	3,56	3,55	3,74	3,4	3,52
7 [mm]	-	3,57	3,7	-		-
8 [mm]	3,65	-	-	-	4,18	-
9 [mm]	3,71	-	3,61	3,7	4,06	2,75
10 [mm]	-	3,62	-	-	3,86	-

Number of replicates	8	8	7	6	8	3
Mean [mm]	3,65	3,65	3,71	3,82	3,86	3,31
Standard deviation	0,05	0,06	0,16	0,12	0,25	0,49
Coefficient of variation %	1,5	1,8	4,4	3,2	6,5	14,8
% Reduction	-	0,1	-1,5	-4,7	-5,8	9,3
Statistical significance	n/d	-	-	-	-	+

Table 9.3-18 Day of the first brood production – definitive test

Concentration [µg/l]	**Control**	**6,4**	**16**	**40**	**100**	**250**
Mean for the concentration	10,5	10,3	10,5	11,7	11,1	10,5
Statistical significance	n/a	-	-	-	-	-

B. OBSERVATIONS

During the preliminary experiment, there was no adults' mortality in the control nor replicates of test item concentrations observed. Adults' body length at the end of the experiment was measured. There was no concentration – dependent impact of the test item observed. There was no concentration – dependent impact of the test item observed. In all tested concentration an increase of offspring number was observed in relation to both – introduced and surviving parent, and in relation to the control. In concentration 100 mg/l the following results were obtained for the offspring number: 120 and 16. Despite the fact that the mean offspring number is higher (68 youngs) than in the control (65 youngs) what indicates lack of ecotoxic properties of test item, it should be taken into consideration that the results were divergent. In accordance with OECD 211 Guideline, two replicates were used, what makes excluding the most digressed result. In view of this and of the fact that the limit test in accordance with OECD Guideline 211 is optional, definitive test was performed in the full range of concentrations and not as a limit test

III. CONCLUSIONS

The test item - BW01 GB demonstrates no toxic effects in terms of survival of parental Daphnia during 21 days of exposure. Also, test item does not influence the survival of offspring daphnias - there was no case of mortality among them observed. Ephippia and male offspring production, and abandonment the eggs are daphnias responses to adverse environmental conditions (eg. presence of toxic substances). The test item shows no negative impact on the listed domains.

It has been shown that the reduction of offspring number produced by the surviving parents at 21st day of the experiment statistically insignificant, thus the test item has no toxic impact on Daphnia reproduction expressed as the offspring number per surviving parents after 21 days of experiment.

Test item demonstrates toxic properties in concentration 250 mg/l in relation to daphnias' growth expressed as their body length of adults at the end of the experiment. Also, it was indicated that test item inhibits reproduction

of Daphnia in concentration 250 mg/l in relation to offspring number per introduced parent and in relation to control.

Results of performed observations and experiment endpoints are shown in Table CP 10.2.2.2.1.

Table 9.3-19 Definitive test endpoints values

Parameter	EC_{10}	EC_{20}	EC_{50}	NOEC	LOEC
Cumulative offspring number per introduced parent (21 days)	215,895 (n/d*)**	225,625 (n/d*)**	245,477 (n/d*)**	100,00	250,000
Cumulative offspring number per survival parent (21 days)	n/d*	n/d*	n/d*	≥ 250,000	> 250,000
Cumulative immobilized offspring number (21 days)	n/d*	n/d*	n/d*	n/d*	n/d*
Cumulative number of abandonment eggs (21 days)	n/d*	n/d*	n/d*	n/d*	n/d*
Całkowita liczba młodych płci męskiej (21 dni)	n/d*	n/d*	n/d*	n/d*	n/d*
Mortality (21 days)****	1,435 (n/d*)**	7,263 (n/d*)**	161,591 (n/d*)**	≥ 250,000	> 250,000
Length of parental specimens	249,779 (16,925 – 3895,033)**	251,093 (0,000 – 733301952,000)**	253,624 (0,000 – 3,17)**	100,00*****	250,00*****
Time of production of the first offspring	n/d*	n/d*	n/d*	n/d*	n/d*

* n/d – not determined due to mathematical reasons of inappropriate data
** lower – upper 95%-CI
***inf – infinity
**** no statistical significance in relation to the control
*****values determined basing on the actual observations, not mathematically

RMS comments:

The Daphnia reproduction study of BW01 GB performed in GLP conditions and according OECD TG 211 is considered valid. Some deviations from the test guideline/study plan were noted, but they are considered as not having significant impact on the study results. All validity criteria were met. The definitive test was performed with a range of nominal concentrations of the test item in 1 L of aqueous medium as a suspension: 6,4 mg/l; 16 mg/l; 40 mg/l; 100 mg/l and 250 mg/l.

The study is considered acceptable with following endpoints:

$NOEC_{reproduction}$ is equal 100 mg product/L as nominal concentration

B.9.3.3 Further testing on aquatic organisms

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

B.9.4 Risk Assessment for Aquatic Organisms

A dossier was submitted for the approval of the active substance ferric pyrophosphate in accordance with Regulation (EC) No 1107/20009, with BW01 GB, a non-dusty and non-volatile granular product containing 30 g/kg (3% w/w) ferric pyrophosphate, as the reference product. The product, which is broadcast in the field over the soil surface, is used as a molluscicide on cereals, oilseed rape and ornamentals. The proposed GAP is presented in Table 1 below.

Table 1 GAP proposed for BW01 GB

Crop	Growth stage	Number of applications	Application interval (days)	Application rate* (kg a.s./ha)	Further information
Edible plants Cereals and oilseed rape	Leaf emergence up to BBCH 15	6	14	0.21	Crops grown in open field
~~Ornamentals~~ Inedible plants	Up to flowering growth stages	6	14	1.5	Crops grown in open field and under covers

* Based on active content of 3% w/w

The pellets are expected to remain on the soil surface until they are eaten by the target pest. Based on evidence from controlled experiments on BW01 GB[1], any pellets not consumed will break down following rainfall or dew. This process takes place from around 3 days after application and up to 2-3 weeks thereafter, depending on rainfall intensity and frequency. The ferric pyrophosphate thus released from the co-formulants in the pellets will be stable and very persistent in the soil due to the compound's low water solubility, data for which is presented at varying pH in Table 2.

Table 2 Water Solubility (µg/l) at 20 ± 0.5°C

pH	24 hours	48 hours	72 hours	Reference
4	140.3	164.8	141.7	M. Włodarczak, 2015; 0001/0164/FA[2]
7	41.2	41.6	39.0	
9	135.9	113.1	112.3	

Following rainfall, however, ferric pyrophosphate residues may be transported to surface water via runoff or drainage. No dust drift is expected during application due the product's non-dusty properties (i.e. 0.0 mg, category 1 of dustiness)[3].

Although significant exposure to aquatic organisms is not expected, an aquatic risk assessment has been carried out in support of the product's evaluation and is presented in this report. It should be noted that a detailed aquatic risk assessment was not required for ferric phosphate, a compound with similar properties to ferric pyrophosphate[4].

2. TOXICITY DATA

Studies on the toxicity to aquatic organisms have been carried out with ferric pyrophosphate. Full details of these studies are provided in the respective EU DAR and related documents. Effects on aquatic organisms of the product BW01 GB were also investigated and summarised in the DAR for the product. No toxicity studies on sediment dwelling organisms were carried out as they were not required[7]. Therefore, the risk assessment for sediment dwelling organisms will be based on the chronic data determined with Daphnia.

The toxicity endpoints relevant for the risk assessment for aquatic organisms are summarised in Tables 3 and 4 below for both the active and formulation, respectively. It should be noted that chronic toxicity data for fish and aquatic invertebrates (Table 4) were available only for the formulation. Toxicity endpoints for chronic effects on aquatic invertebrates were determined on the basis of nominal concentrations only.

Table 3 Toxicity endpoints determined for aquatic organisms – Ferric Pyrophosphate

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Ferric pyrophosphate	96 h, s	**LC_{50} >0.134 mg a.s./L_{im}** (>100 mg/L_{nom})	[redacted] (2013a 0003/0024/E[5]
Daphnia magna	Ferric pyrophosphate	48 h, s	**EC_{50} >0.092 mg a.s./L_{im}** (>100 mg/L_{nom})	Ziółkowska, A. and Wickiel, G., (2013b 0003/0022/E[6]
Chironomus riparius	-	-	No studies carried out	DAR, Volume 3-B.9, January 2018[7]
Pseudokirchneriella subcapitata	Ferric pyrophosphate	72 h, s	**E_rC_{50} >0.021 mg a.s./L_{im} E_yC_{50} >0.021 mg a.s./L_{im}** (>100 mg/L_{nom})	Heisterkamp I. 2015, 1040[8]

s: static; nom: based on nominal concentrations; im: based on initial measured concentrations

Table 4 Toxicity endpoints determined for aquatic organisms for aquatic organisms – Formulation BW01 GB

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	BW01 GB	96 h, ss	**(LC_{50} >0.222 mg a.s./L)** LC_{50} >7.4 mg product/L_{mm} (>100 mg product/L_{nom})	[redacted].(2014a), No. 0001/0091/E[9]
Danio rerio	BW01 GB	30-day (Long-term and chronic toxicity)	**NOEC =0.138 mg a.s./L** NOEC=4.6 mg product/L - measured concentration (10 mg product/L_{nom})	[redacted].(2014b), No. 0001/0109/E[10]
Daphnia magna	BW01 GB	48 h, s	**EC_{50} >0.212 mg a.s./L** EC_{50} >7.055 mg product/L_{mm} (>100 mg product/L_{nom})	Winkler J. (2014a), No. 0001/0090/E[11]
Daphnia magna	BW01 GB	21-days, ss	**NOECreproduction =3 mg a.s./L_{nom}** NOECreproduction =100 mg product/L_{nom}	Winkler J. (2014b), 0001/0111/E[12]

s: static; ss: semi-static; nom: based on nominal concentrations; mm: based on mean measured concentrations

9.4.1. RISK ASSESSMENT FOR SURFACE WATER

Exposure

Entry of the substance into surface water following broadcast application of the BW01 GB pellets can potentially occur via drainage and runoff following release of the substance into the soil. Predicted Environmental Concentrations for surface water (PECsw) were determined for both of these routes using FOCUS models for drainage/runoff.[13]

Risk Assessment with toxicity endpoints based on measured concentrations

The evaluation of the risk for aquatic organisms was performed in accordance with the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009"[14].

The risk assessment was carried out using the Regulatory Acceptable Concentration (RAC) approach. Firstly, RACs were determined for each organism using the toxicity data available for the active substance and formulation. The RAC for each organism was determined by dividing the relevant toxicity endpoint by the assessment factor (AF) (i.e. 100 for acute effects and 10 for chronic effects).

In the following tables, the ratios between PEC_{SW} and RAC values for aquatic organisms are given per intended use for each FOCUS scenario and each organism group. The resulting PEC/RAC ratios above the relevant trigger of 1 are shown in bold. For the intended uses of arable crops and ornamentals, calculated PEC/RAC ratios did not indicate an acceptable risk for the species tested in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies. These values are also presented in the tables below for each crop group investigated.

~~**Arable Crops**~~

Edible Plants

Table 5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ferric pyrophosphate for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of BW01 GB in arable crops (6 x 210 g/ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Danio rerio***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint (µg/L)		LC_{50} >134	NOEC 138	EC_{50} >92	E_rC_{50}/E_yC_{50} >21.2
AF		100	10	100	10
RAC (µg/L)		1.34	13.8	0.92	2.12
FOCUS Scenario	**PEC sw-max** (µg/L)	**PEC/RAC**			
Step 1					
	29.3	**21.9**	**2.12**	**31.8**	**13.8**
Step 2					
N-Europe *(Oct-Feb)*	14.3	**10.6**	**1.03**	**15.5**	**6.73**
N-Europe *(Mar-May)*	5.71	**4.26**	0.414	**6.21**	**2.69**
S-Europe	11.4	**8.51**	0.83	**12.4**	**5.38**

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Step 3					
D1/ditch	0.150	0.112	0.011	0.163	0.071
D1/stream	0.104	0.078	0.008	0.113	0.049
D2/ditch	19.4	**14.5**	**1.40**	**21.1**	**9.14**
D2/stream	12.2	**9.07**	0.881	**13.2**	**5.74**
D3/ditch	0.000	0.000	0.000	0.000	0.000
D4/pond	0.351	0.262	0.025	0.382	0.166
D4/stream	1.786	**1.33**	0.129	**1.94**	0.84
D5/pond	0.095	0.071	0.007	0.103	0.045
D5/stream	0.768	0.573	0.056	0.835	0.362
D6/ditch	14.73	**10.99**	**1.07**	**16.0**	**6.95**
R1/pond	0.390	0.291	0.028	0.424	0.184
R1/stream	16.6	**12.4**	**1.20**	**18.0**	**7.81**
R2/stream	-	-	-	-	-
R3/stream	22.2	**16.6**	**1.61**	**24.2**	**10.49**
R4/stream	13.3	**9.96**	0.967	**14.5**	**6.29**
Step 4					
Scenario (Vegetative Buffer Strip 10 m)					
R1 pond	0.157	0.117	0.011	0.171	0.074
R1 stream	7.52	**5.61**	0.545	**8.17**	**3.55**
R3 stream	9.86	**7.36**	0.715	**10.7**	**4.65**
R4 stream	5.93	**4.43**	0.430	**6.45**	**2.80**
Scenario (Vegetative Buffer Strip 20 m)					
R1 pond	0.079	0.059	0.006	0.086	0.037
R1 stream	3.94	**2.94**	0.286	**4.28**	**1.86**
R3 stream	5.12	**3.82**	0.371	**5.56**	**2.41**
R4 stream	3.08	**2.30**	0.223	**3.35**	**1.45**

The risk assessment for arable crops failed for all aquatic species at Steps 1 and 2 for the scenarios investigated, with the exception of the chronic risk assessment for fish, which passed at Steps 2 Northern Europe (March-May) and Southern Europe.

At Steps 3, the acute and chronic risk assessments for arable crops was acceptable for all aquatic organisms for the drainage scenarios D1/ditch, D1/stream, D3/ditch, D5/pond and D5 stream. With the exception of the chronic risk assessment for fish, the D2/stream scenario failed all risk assessments. The D4/stream failed the acute aquatic risk assessments for fish and aquatic invertebrates.

In terms of runoff, the R1/pond scenario passed all risk assessments. The risk assessment for aquatic species was unacceptable (i.e. PEC/RAC>1) for the R1 stream scenario. Step 4 only helped mitigation with respect to the chronic risk assessment.

~~**Ornamentals**~~

Inedible plants

Table 6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ferric pyrophosphate for each organism group based on FOCUS Steps 1, 2, 3 and 4 calculations for the use of BW01 GB in ornamentals (6 x 1500 g/ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	**Zebrafish (*Danio rerio*)**	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint (µg/L)		LC_{50} >134	NOEC 138	EC_{50} >92	E_rC_{50}/E_yC_{50} >21.2
AF		100	10	100	10
RAC (µg/L)		1.34	13.8	0.92	2.12
FOCUS Scenario	**PEC sw-max (µg/L)**	**PEC/RAC**			
Step 1					
	209.3	**156.2**	**15.2**	**227.5**	**98.7**
Step 2					
N-Europe *(Oct-Feb)* N-Europe *(Mar-May)*	101.9 40.75	**76.0** **30.4**	**7.38** **2.95**	**110.8** **44.3**	**48.1** **19.2**
S-Europe	81.5	**60.8**	**5.91**	**88.6**	**38.4**
Step 3					
D1/ditch	-	-	-	-	-
D1/stream	-	-	-	-	-
D2/ditch	-	-	-	-	-
D2/stream	-	-	-	-	-
D3/ditch	0.000	0.000	0.000	0.000	0.000
D4/pond	5.55	**4.14**	0.402	**6.04**	**2.62**
D4/stream	20.9	**15.6**	**1.52**	**22.8**	**9.87**

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
D5/pond	-	-	-	-	-
D5/stream	-	-	-	-	-
D6/ditch	72.2	**53.8**	**5.23**	**78.4**	**34.0**
R1/pond	10.8	**8.08**	0.785	**11.8**	**5.11**
R1/stream	121.1	**90.4**	**8.78**	**131.6**	**57.1**
R2/stream	61.3	**45.7**	**4.44**	**66.6**	**28.9**
R3/stream	75.6	**56.4**	**5.48**	**82.2**	**35.7**
R4/stream	**71.4**	**53.3**	**5.17**	**77.6**	**33.7**

Step 4

Scenario (Vegetative Buffer Strip 10 m)					
R1 pond	4.36	**3.26**	0.316	**4.74**	**2.06**
R1 stream	55.2	**41.2**	**4.00**	**60.0**	**26.0**
R2 stream	27.9	**20.8**	**2.02**	**30.4**	**13.2**
R3 stream	34.5	**25.7**	**2.50**	**37.5**	**16.3**
R4 stream	31.4	**23.5**	**2.28**	**34.2**	**14.8**
Scenario (Vegetative Buffer Strip 20 m)					
R1 pond	2.19	**1.63**	0.159	**2.38**	**1.03**
R1 stream	28.9	**21.6**	**2.10**	**31.4**	**13.6**
R2 stream	14.7	**10.9**	**1.06**	**15.9**	**6.91**
R3 stream	18.1	**13.5**	**1.31**	**19.7**	**8.53**
R4 pond	16.5	**12.3**	**1.19**	**17.9**	**7.76**

The risk assessment for ornamentals failed for all aquatic species at Steps 1 and 2 for the scenarios investigated.

At Steps 3 for inedible plants (ornamentals), all scenarios failed the risk assessments, with the exception of the D3/ditch scenarios. Only D3/ditch, D4/pond, and R1/pond scenarios passed the chronic risk assessment for fish. At Step 4, the R1/pond scenario failed the aquatic risk assessment at both 10 m and 20 m vegetative buffer zone in contrast to Step 3. The other scenarios also failed the risk assessments at Step 4.

The aquatic risk assessment carried out for the granular product BW01 GB based on toxicity endpoints determined for measured concentrations is not appropriate as the toxicity values proposed in the studies are too low and do

not provide a realistic representation of the product's toxicity profile. These values were based on the solubility limit of the substance. A review of the toxicity aquatic studies indicates that only a small percentage of ferric pyrophosphate could be analytically measured compared to the nominal concentrations used. Therefore, ferric pyrophosphate is a difficult test substance for aquatic experimental testing given its low solubility in water.

Weyman et. al. (2012)[15] has reviewed the limitations of aquatic toxicity testing with substances that are poorly soluble in water and discusses the consequences for the environmental aquatic risk assessment. Analytical measurements of exposure concentrations should reflect what organisms are exposed to. If no adverse effects occur at the saturation limit, the risk should be acceptable because higher aqueous exposure cannot occur. Assessment factors for NOEC values at the saturation limit require careful consideration in the risk assessment to avoid unnecessarily low regulatory acceptable concentrations. There is a suggestion of the possibility of lowering the assessment factor to one. If this was the case, the risk assessment for all aquatic species would be acceptable at Step 4, implying there would be no risk at the water solubility limit.

~~A risk assessment based on toxicity endpoints obtained from nominal concentrations is outlined in the next section.~~

~~Risk Assessment with toxicity endpoints based on nominal concentrations~~

~~Results of the aquatic risk assessment based on toxicity endpoints based on nominal concentrations are presented below for the intended uses of BW01 GB for arable crops and ornamentals in Tables 7 and 8, respectively.~~

~~Table 7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ferric pyrophosphate for each organism group based on FOCUS Steps 1-4 calculations for the use of BW01 GB in arable crops (6 x 210 g/ha)~~

~~Group~~		~~Fish acute~~	~~Fish prolonged~~	~~Inverteb. acute~~	~~Inverteb. prolonged~~	~~Algae~~
~~Test species~~		~~*Oncorhynchus mykiss*~~	~~*Danio rerio*~~	~~*Daphnia magna*~~	~~*Daphnia magna*~~	~~*Pseudokirchn. subcapitata*~~
~~Endpoint (µg/L)~~		~~LC_{50} >100,000~~	~~NOEC 10,000~~	~~EC_{50} >100,000~~	~~NOEC 3,000~~	~~E_rC_{50}/E_yC_{50} >100,000~~
~~AF~~		~~100~~	~~10~~	~~100~~	~~10~~	~~10~~
~~RAC (µg/L)~~		~~1,000~~	~~1,000~~	~~1,000~~	~~300~~	~~10,000~~
~~FOCUS Scenario~~	~~PEC sw-max (µg/L)~~	~~PEC/RAC~~				
~~Step 1~~						
	~~29.3~~	~~0.029~~	~~0.029~~	~~0.029~~	~~0.098~~	~~0.003~~
~~Step 2~~						
~~N Europe *(Oct-Feb)*~~ ~~N Europe *(Mar-May)*~~	~~14.3~~ ~~5.71~~	~~0.014~~ ~~0.006~~	~~0.014~~ ~~0.006~~	~~0.014~~ ~~0.006~~	~~0.048~~ ~~0.019~~	~~0.001~~ ~~0.001~~
~~S Europe~~	~~11.4~~	~~0.011~~	~~0.011~~	~~0.011~~	~~0.038~~	~~0.001~~
~~Step 3~~						
~~D1/ditch~~	~~0.150~~	~~0.0002~~	~~0.0002~~	~~0.0002~~	~~0.001~~	~~0.00002~~

~~Group~~		~~Fish acute~~	~~Fish prolonged~~	~~Inverteb. acute~~	~~Inverteb. prolonged~~	~~Algae~~
~~D1/stream~~	~~0.104~~	~~0.0001~~	~~0.0001~~	~~0.0001~~	~~0.0003~~	~~1.04E-05~~
~~D2/ditch~~	~~19.370~~	~~0.019~~	~~0.019~~	~~0.019~~	~~0.065~~	~~0.002~~
~~D2/stream~~	~~12.160~~	~~0.012~~	~~0.012~~	~~0.012~~	~~0.041~~	~~0.001~~
~~D3/ditch~~	~~0.000~~	~~0~~	~~0~~	~~0~~	~~0~~	~~0~~
~~D4/pond~~	~~0.351~~	~~0.0004~~	~~0.0004~~	~~0.0004~~	~~0.001~~	~~3.51E-05~~
~~D4/stream~~	~~1.786~~	~~0.002~~	~~0.002~~	~~0.002~~	~~0.006~~	~~0.0002~~
~~D5/pond~~	~~0.095~~	~~0.0001~~	~~0.0001~~	~~0.0001~~	~~0.0003~~	~~9.5E-06~~
~~D5/stream~~	~~0.768~~	~~0.0008~~	~~0.0008~~	~~0.0008~~	~~0.003~~	~~7.68E-05~~
~~D6/ditch~~	~~14.73~~	~~0.015~~	~~0.015~~	~~0.015~~	~~0.049~~	~~0.001~~
~~R1/pond~~	~~0.390~~	~~0.0004~~	~~0.0004~~	~~0.0004~~	~~0.001~~	~~0.00004~~
~~R1/stream~~	~~16.560~~	~~0.017~~	~~0.017~~	~~0.017~~	~~0.055~~	~~0.002~~
~~R2/stream~~	~~-~~	~~–~~	~~–~~	~~–~~	~~–~~	~~–~~
~~R3/stream~~	~~22.240~~	~~0.022~~	~~0.022~~	~~0.002~~	~~0.074~~	~~0.002~~
~~R4/stream~~	~~13.340~~	~~0.013~~	~~0.013~~	~~0.013~~	~~0.044~~	~~0.001~~

~~**Step 4**~~

~~**Scenario (Vegetative Buffer Strip 10 m)**~~						
~~R1 pond~~	~~0.157~~	~~0.0002~~	~~0.0002~~	~~0.0002~~	~~0.001~~	~~0.00002~~
~~R1 stream~~	~~7.52~~	~~0.008~~	~~0.008~~	~~0.008~~	~~0.025~~	~~0.001~~
~~R3 stream~~	~~9.86~~	~~0.010~~	~~0.010~~	~~0.010~~	~~0.033~~	~~0.001~~
~~R4 stream~~	~~5.93~~	~~0.006~~	~~0.006~~	~~0.006~~	~~0.020~~	~~0.001~~
~~**Scenario (Vegetative Buffer Strip 20 m)**~~						
~~R1 pond~~	~~0.079~~	~~0.0001~~	~~0.0001~~	~~0.0001~~	~~0.0003~~	~~7.9E-06~~
~~R1 stream~~	~~3.94~~	~~0.004~~	~~0.004~~	~~0.004~~	~~0.013~~	~~0.0004~~
~~R3 stream~~	~~5.116~~	~~0.005~~	~~0.005~~	~~0.005~~	~~0.017~~	~~0.001~~
~~R4 stream~~	~~3.078~~	~~0.003~~	~~0.003~~	~~0.003~~	~~0.010~~	~~0.0003~~

~~**Table 8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ferric pyrophosphate for each organism group based on FOCUS Steps 1-4 calculations for the use of BW01-GB in ornamentals (6 x 1500 g/ha)**~~

~~**Group**~~		~~**Fish acute**~~	~~**Fish prolonged**~~	~~**Inverteb. Acute**~~	~~**Inverteb. prolonged**~~	~~**Algae**~~
~~**Test species**~~		~~***Oncorhynchus mykiss***~~	~~**Zebrafish (*Danio rerio*)**~~	~~***Daphnia magna***~~	~~***Daphnia magna***~~	~~***Pseudokirchn. subcapitata***~~
~~**Endpoint (µg/L)**~~		~~LC_{50} >100,000~~	~~NOEC 10,000~~	~~EC_{50} >100,000~~	~~NOEC 3,000~~	~~E_rC_{50}/E_yC_{50} >100,000~~
~~**AF**~~		~~100~~	~~10~~	~~100~~	~~10~~	~~10~~
~~**RAC (µg/L)**~~		~~1,000~~	~~1,000~~	~~1,000~~	~~300~~	~~10,000~~
~~**FOCUS Scenario**~~	~~**PEC sw-max (µg/L)**~~	~~**PEC/RAC**~~				
~~**Step 1**~~						
	~~209.3~~	~~0.209~~	~~0.209~~	~~0.209~~	~~0.698~~	~~0.021~~
~~**Step 2**~~						
~~N Europe *(Oct-Feb)*~~ ~~N Europe *(Mar-May)*~~	~~101.9~~ ~~40.75~~	~~0.102~~ ~~0.041~~	~~0.102~~ ~~0.041~~	~~0.102~~ ~~0.041~~	~~0.340~~ ~~0.136~~	~~0.010~~ ~~0.004~~
~~S Europe~~	~~81.5~~	~~0.082~~	~~0.082~~	~~0.082~~	~~0.272~~	~~0.008~~
~~**Step 3**~~						
~~D1/ditch~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~D1/stream~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~D2/ditch~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~D2/stream~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~D3/ditch~~	~~0.000~~	~~0.000~~	~~0.000~~	~~0.000~~	~~0.000~~	~~0.000~~
~~D4/pond~~	~~5.55~~	~~0.006~~	~~0.006~~	~~0.006~~	~~0.019~~	~~0.001~~
~~D4/stream~~	~~20.9~~	~~0.021~~	~~0.021~~	~~0.021~~	~~0.070~~	~~0.002~~
~~D5/pond~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~D5/stream~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~
~~D6/ditch~~	~~72.2~~	~~0.072~~	~~0.072~~	~~0.072~~	~~0.241~~	~~0.007~~
~~R1/pond~~	~~10.8~~	~~0.011~~	~~0.011~~	~~0.001~~	~~0.036~~	~~0.001~~
~~R1/stream~~	~~121.1~~	~~0.121~~	~~0.121~~	~~0.121~~	~~0.404~~	~~0.012~~
~~R2/stream~~	~~61.3~~	~~0.061~~	~~0.061~~	~~0.061~~	~~0.204~~	~~0.006~~
~~R3/stream~~	~~75.6~~	~~0.076~~	~~0.076~~	~~0.076~~	~~0.252~~	~~0.008~~
~~R4/stream~~	~~71.4~~	~~0.071~~	~~0.071~~	~~0.071~~	~~0.238~~	~~0.007~~
~~**Step 4**~~						
~~**Scenario (Vegetative Buffer Strip 10 m)**~~						
~~R1 pond~~	~~4.364~~	~~0.004~~	~~0.004~~	~~0.004~~	~~0.015~~	~~0.0004~~

~~**Group**~~		~~**Fish acute**~~	~~**Fish prolonged**~~	~~**Inverteb. Acute**~~	~~**Inverteb. prolonged**~~	~~**Algae**~~
~~R1 stream~~	~~55.18~~	~~0.055~~	~~0.055~~	~~0.055~~	~~0.184~~	~~0.006~~
~~R2 stream~~	~~27.93~~	~~0.028~~	~~0.028~~	~~0.028~~	~~0.093~~	~~0.003~~
~~R3 stream~~	~~34.49~~	~~0.034~~	~~0.034~~	~~0.003~~	~~0.115~~	~~0.003~~
~~R4 stream~~	~~31.44~~	~~0.031~~	~~0.031~~	~~0.031~~	~~0.105~~	~~0.003~~
~~**Scenario (Vegetative Buffer Strip 20 m)**~~						
~~R1 pond~~	~~2.188~~	~~0.002~~	~~0.002~~	~~0.002~~	~~0.007~~	~~0.0002~~
~~R1 stream~~	~~28.93~~	~~0.029~~	~~0.029~~	~~0.029~~	~~0.096~~	~~0.003~~
~~R2 stream~~	~~14.65~~	~~0.015~~	~~0.015~~	~~0.015~~	~~0.049~~	~~0.001~~
~~R3 stream~~	~~18.09~~	~~0.018~~	~~0.018~~	~~0.018~~	~~0.060~~	~~0.002~~
~~R4 stream~~	~~16.45~~	~~0.016~~	~~0.016~~	~~0.016~~	~~0.055~~	~~0.002~~

~~The PEC/RAC values for all of the above FOCUS scenarios are below 1 indicating the risk for aquatic organisms is acceptable when using toxicity endpoints based on nominal concentrations.~~

9.4.2 . RISK ASSESSMENT FOR SEDIMENT

9.4.2.1 Exposure

Entry of the substance into sediment following broadcast application of the BW01 GB pellets can potentially occur via drainage and runoff following release of the substance into the soil. Predicted Environmental Concentrations for sediment (PECsed) were determined for both of these routes using FOCUS models for drainage/runoff.[13]

9.4.2.2 Risk Assessment

No toxicity studies are available for sediment dwelling organisms, and they are not required due to lack of insecticidal activity of Ferric Pyrophosphate

~~The resulting PECsed/RAC ratios are presented in the Tables 9 and 10 for arable crops and ornamentals, respectively, for the different FOCUS scenarios. Those values found to be above the relevant trigger of 1 are shown in bold.~~

~~**Table 9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ferric pyrophosphate for each organism group based on FOCUS Steps 1-4 calculations for the use of BW01 GB in arable crops (6 x 210 g/ha)**~~

~~**Group**~~		~~**Inverteb. Prolonged**~~
~~**Test species**~~		~~***Daphnia magna***~~
~~**Endpoint (µg/L)**~~		~~NOEC 3,000~~
~~**AF**~~		~~10~~
~~**RAC (µg/L)**~~		~~300~~
~~**FOCUS Scenario**~~	~~**PEC sed max (µg/L)**~~	~~**PEC/RAC**~~
~~**Step 1**~~		
	~~2930~~	~~**9.77**~~
~~**Step 2**~~		
~~N Europe *(Oct Feb)*~~ ~~N Europe *(Mar May)*~~	~~1430~~ ~~570.1~~	~~**4.77**~~ ~~**1.90**~~
~~S Europe~~	~~1140~~	~~**3.80**~~
~~**Step 3**~~		
~~D1/ditch~~	~~0.066~~	~~0.0002~~
~~D1/stream~~	~~0.041~~	~~0.0001~~
~~D2/ditch~~	~~88.6~~	~~0.295~~
~~D2/stream~~	~~36.5~~	~~0.122~~
~~D3/ditch~~	~~0.000~~	~~0~~
~~D4/pond~~	~~3.00~~	~~0.010~~
~~D4/stream~~	~~1.49~~	~~0.005~~
~~D5/pond~~	~~1.21~~	~~0.004~~
~~D5/stream~~	~~0.191~~	~~0.001~~
~~D6/ditch~~	~~5.83~~	~~0.019~~
~~R1/pond~~	~~6.72~~	~~0.022~~

~~R1/stream~~	~~6.57~~	~~0.022~~
~~R2/stream~~	~~-~~	~~-~~
~~R3/stream~~	~~18.47~~	~~0.062~~
~~R4/stream~~	~~8.94~~	~~0.030~~

~~**Table 10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ferric pyrophosphate for each organism group based on FOCUS Steps 1-4 calculations for the use of BW01 GB in ornamentals (6 x 1500 g/ha)**~~

~~**Group**~~		~~**Inverteb. Prolonged**~~
~~**Test species**~~		~~***Daphnia magna***~~
~~**Endpoint**~~		~~NOEC~~
~~**(µg/L)**~~		~~3,000~~
~~**AF**~~		~~10~~
~~**RAC (µg/L)**~~		~~300~~
~~**FOCUS Scenario**~~	~~**PEC sed max (µg/L)**~~	~~**PEC/RAC**~~
~~**Step 1**~~		
	~~20900~~	~~**69.7**~~
~~**Step 2**~~		
~~N-Europe *(Oct-Feb)*~~	~~10200~~	~~**34.0**~~
~~N-Europe *(Mar-May)*~~	~~4070~~	~~**13.6**~~
~~S-Europe~~	~~8150~~	~~**27.2**~~
~~**Step 3**~~		
~~D1/ditch~~	~~-~~	~~-~~
~~D1/stream~~	~~-~~	~~-~~
~~D2/ditch~~	~~-~~	~~-~~
~~D2/stream~~	~~-~~	~~-~~
~~D3/ditch~~	~~0.000~~	~~0.000~~
~~D4/pond~~	~~49.7~~	~~0.166~~
~~D4/stream~~	~~25.0~~	~~0.083~~
~~D5/pond~~	~~-~~	~~-~~
~~D5/stream~~	~~-~~	~~-~~
~~D6/ditch~~	~~161.8~~	~~0.539~~
~~R1/pond~~	~~159.9~~	~~0.533~~
~~R1/stream~~	~~52.3~~	~~0.174~~
~~R2/stream~~	~~81.1~~	~~0.270~~
~~R3/stream~~	~~62.8~~	~~0.209~~
~~R4/stream~~	~~89.4~~	~~0.298~~

~~When using a chronic endpoint determined with Daphnia as a surrogate for an endpoint for sediment dwelling organisms, the above risk assessment is demonstrated as acceptable considering higher tier FOCUS modelling at Steps 3. No risk mitigations are needed.~~

In addition to this, the iron and phosphate levels that occur naturally in sediment are expected to be many orders of magnitude higher than those that result from application of BW01 GB in the field. On this basis, it is unlikely that ferric pyrophosphate released from the use of BW01 GB will have a negative impact on sediment dwelling organisms since these non-target organisms are naturally exposed to iron and phosphate ions in the environment.

5. CONCLUSIONS

The aquatic risk assessment carried out for the granular product BW01 GB based on measured concentrations is not appropriate as the toxicity values proposed in the studies are too low. These values, which are based on the results for low solubility limit of ferric pyrophosphate, result in unnecessarily low regulatory acceptable concentrations causing the risk assessment to fail.

All studies were conducted with a nominal concentration of active of 100 mg/L and no effects were observed at this limit regardless of solubility issues. ~~Safe use is demonstrated when the risk assessment is based on nominal values.~~ Therefore, it is proposed that the aquatic risk assessment is acceptable and there should be no concerns when the product is used in the field at the recommended application rates.

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RMS: Neither ferric pyrophosphate nor a product BW01 GB have induced any acute, chronic or reproduction toxic effect in any taxon of aquatic organisms in the standard acceptable studies in which an active substance was at the measured concentrations very close to or equal to the level of its solubility in water, and nominal concentrations of ferric pyrophosphate were up to 100mg/L. Thus the exact values acute or chronic toxicity endpoints could not be determined. The results of these studies demonstrate that the nominal values of endpoints (LC50, NOEC, ErLR50 and EyLR50) if exist, would be well above the top doses or levels foreseen in the internationally recognised test method. Summing up the results of these studies provide evidence that the ferric pyrophosphate and product BW01 GB does not warrant classification for environmental hazards according to criteria of Regulation (EU) 1272/2008, therefore the assessment of risk might not be determined using standard approaches due to lack of evidence of aquatic toxicity .

Due to the above explained reasons the quantitative assessment of risk for aquatic organisms must be interpreted very carefully, although a calculated ratio of PEC/RAC takes into account Regulatory Acceptable Concentration (RAC) determined in line with "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009" but using toxicity endpoints (LC50, NOEC, ErLR50 and EyLR50) which were practically not determined due to low toxicity of ferric pyrophosphate. So all the estimated values of PEC/RAC does not have toxicological or risk assessment meaning, since RAC values are based on the highest achievable concentrations of ferric pyrophosphate in water not causing any toxic effect. In none of the studies any toxic effect in aquatic organisms were seen.

In this situation, for practical reasons, it is appropriate to base an assessment of risk posed by ferric pyrophosphate or a product BW01 GB to aquatic organisms on information derived for the standard tests~~. using a nominal concentrations used in them.~~ These studies demonstrate that at a high load of water with ferric pyrophosphate, by far greater than the load of surface water with ferric pyrophosphate due to application of a product BW01 GB in line with GAP no toxic effects were seen.

Therefore, the existing data provide evidence that application of a product BW01 GB in line with GAP does not pose an unacceptable risk to aquatic organisms.

Overall conclusion regarding risk to aquatic organisms:

No unacceptable acute and chronic risk to aquatic organisms is anticipated when formulation BW01 GB is applied according to proposed use pattern (1 to 6 times per season x 0.12 - 1.5 kg a.s./ha with minimum interval between applications is 2-4 weeks). No risk mitigation measures are required.

B.9.5 Effects on Arthropods

B.9.5.1 Effects on bees

B.9.5.1 Supplementary assessment of effects on bees

Introduction

BW01 GB is a non-dusty and non-volatile granular bait product for use as a molluscicide on cereals, oilseed rape and ornamentals. The product contains 30 g/kg (3% w/w) of ferric pyrophosphate as an active substance. The product is broadcast in the field over the soil surface.

The table below shows the Good Agricultural Practice (GAP) for the intended uses of BW01 GB.

Table 1. GAP proposed for BW01 GB

Crop	Growth stage	Number of applications	Application interval (days)	Application rate* (kg a.s./ha)	Further information
Cereals and oilseed rape	Leaf emergence up to BBCH 15	6	14	0.21	Crops grown in open field
Ornamentals	Up to flowering growth stages	6	14	1.5	Crops grown in open field and under covers

* Based on active content of 3% w/w

The toxicity data determined with honey bees for ferric pyrophosphate are presented in the following table below.

Table 2. Toxicity data with honey bees for ferric pyrophosphate

Test substance	Toxicity endpoints	Study report
Ferric pyrophosphate	48 h oral LD 50 > 100 µg a.s./bee	Ziółkowska, A. (2013) Study code: 0003/0026/E
	48 h contact LD50 > 100 µg a.s./bee	Winkler, J. (2013) Study code: 0003/0025/E

The risk to bees from dust drift during application should be considered for granular products.

However, it is expected that BW01 GB will not pose a risk to bees based on the negligible dust drift expected following the application of BW01 GB, due to its non-dusty properties (i.e. 0.0 mg, category 1 of dustiness). In addition, it is very unlikely that bees will be attracted to ornamentals during BW01 GB application as the applications will be carried out before the flowering growth stage (Table 1). Moreover, for another non-dusty granular product containing ferric phosphate (a very similar active substance to ferric pyrophosphate), a quantitative risk assessment was not considered necessary to demonstrate an acceptable risk to bees (3).

General considerations for the risk assessment

The risk assessment was conducted in line with the new EFSA guidance document on the risk assessment of plant protection products (5). Although this guidance document has not been officially noted to date, it covers the risk for granular applications that are not currently considered under the SANCO guidance document (6) .

Oral and contact toxicity tests were performed with honey bees for ferric pyrophosphate (Table 2). The risk to other species such as bumble bees and solitary bees should also be considered in the risk assessment. When there is no toxicity data for bumble bees and solitary bees, the new EFSA guidance recommends using the honey bee toxicity endpoint as surrogate with an assumption of 10-fold greater toxicity as a conservative approach (5). Therefore, the toxicity values reported in Table 2 were divided by a safety factor of 10 and used as toxicity endpoints in the risk assessment for bumble bees and solitary bees.

The risk to bees from contact and oral exposures has been assessed. It should be noted that ornamentals could not be allocated under any of the crop categories established by the new EFSA guidance (5) for the risk assessment. The leafy vegetables category was used as surrogate for ornamentals, as a similar approach was used by the new EFSA guidance (5). In the contact risk assessment, it should be specified whether the applications will be carried out during flowering of an attractive crop. For cereals and oilseed rape uses, it was assumed that the application was done during flowering; whereas for ornamentals, the application was not conducted during flowering in line with the GAP for BW01 GB (Table 1). The risk to bees through drinking contaminated water was also assessed using the toxicity values presented in Table 2 and the following input values outlined in Table 3. These values were extracted from the report of the environmental behaviour of ferric pyrophosphate following application of BW01 GB (7). As a conservative approach, the maximum Predicted Exposure Concentration in surface water (PEC sw) values calculated at FOCUS Step 1 for each crop were used as surrogate values for PEC run-off. At Step 1 inputs of spray drift, run-off, erosion and/or drainage are evaluated as a single loading to the water body and "worst-case" surface water and sediment concentrations are calculated.

Table 3. Inputs used in the risk assessment for exposure to contaminated water

Test substance	Input parameter (7) (g a.s./L)	Cereals and oilseed rape	Ornamentals
Ferric pyrophosphate	Water solubility	4 x 10-5	
	Max PEC sw#	7 x 10-5	5 x 10-4
	Max PEC run-off *	3 x 10-5	2 x 10-4

PEC sw : Predicted Exposure Concentration in surface water

Max PEC sw calculated following a single direct application to surface water

* Maximum PEC sw values calculated at FOCUS Step 1

Acute risk assessment for contact exposure

The contact exposure to the dust originating from the granules deposited on the treated crop, attractive weeds and field margins are considered in the contact risk assessment. This assessment is based on the Hazard Quotient (HQ). The tables below show the results of the contact risk assessment following the granular application of BW01 GB according to the recommended GAP (Table 1).

Table 4. Contact risk assessment following granular application for cereals and oilseed rape

Crop category	Scenario	BBCH	f dep	Honey bee		Bumble bee#		Solitary bee#	
				HQ	Trigger	HQ	Trigger	HQ	Trigger
Cereals and oilseed rape	treated crop	< 10	0	N/A	N/A	N/A	N/A	N/A	N/A
		10 - 29	0.1	0.2	14	2.1	2.3	2.1	2.6
	weeds	< 10	0	N/A	N/A	N/A	N/A	N/A	N/A
		10 - 29	0.1	0.2	14	2.1	2.3	2.1	2.6
	field margin	< 10	0.03	0.2	14	2.0	2.3	2.0	2.6
		10 - 29	0.096	0.2	14	2.0	2.3	2.0	2.6

HQ: Hazard Quotient; f dep : deposition factor; N/A: Not applicable; BBCH: Growth stage

#The honey bee toxicity value divided by a safety factor of 10 was used for these bee species

For cereals and oilseed rape, the HQ values calculated for the treated crop, weeds and field margin scenarios for all bee species were below the respective trigger values indicating the contact risk to bees from the use of BW01 GB is acceptable.

Table 5. Contact risk assessment following granular application for ornamentals

Crop category*	Scenario	BBCH	f dep	Honey bee		Bumble bee#		Solitary bee#	
				HQ	Trigger	HQ	Trigger	HQ	Trigger
Leafy vegetables	treated crop	< 10	0	N/A	N/A	N/A	N/A	N/A	N/A
		10 - 49	0	0.0	14	0.0	2.3	0.0	2.6
		≥ 50	0	0.0	14	0.0	2.3	0.0	2.6
	weeds	< 10	0	N/A	N/A	N/A	N/A	N/A	N/A
		10 - 49	0.1	1.5	14	15.0	2.3	15.0	2.6
		≥ 50	0.03	0.5	14	4.5	2.3	4.5	2.6
	field margin	< 10	0.096	1.4	14	14.4	2.3	14.4	2.6
		10 - 49	0.096	1.4	14	14.4	2.3	14.4	2.6
		≥ 50	0.096	1.4	14	14.4	2.3	14.4	2.6

HQ: Hazard Quotient; f dep : deposition factor; N/A: Not applicable; BBCH: Growth stage

*Leafy vegetables crop category used as surrogate for ornamentals

#The honey bee toxicity value divided by a safety factor of 10 was used for these bee species

Bold HQ values identify a potential risk from BW01 GB application

For ornamentals, the HQ values for honey bees for the three risk scenarios assessed were below the trigger value of 14, indicating the risk to this bee species is acceptable from the use of BW01 GB. The risk to bumble bees and solitary bees on the treated crop is also considered to be acceptable. However, the HQ values calculated for bumble bees and solitary bees for weeds at BBCH > 10 and the field margin scenarios were above their respective trigger values. The unacceptable risk to bumble bees and solitary bees can be explained by the double conservative assumptions used in the risk assessment for these species (i.e. the 10-fold higher toxicity and the lower trigger values compared to those used for honey bees, Table 5). Since little research has been carried out to investigate the toxic sensitivities of bumble bees and solitary bees to pesticide active substances, much lower trigger values are considered in the risk assessment for these species (5). In addition, ornamentals will be grown under covers (Table 1), which would result in a negligible dust drift from the treated crop.

Acute risk assessment for oral exposure

The risk from oral exposure following granular applications should also be assessed. The oral exposure assessment for both spray and solid formulations is based on the Exposure Toxicity Ratio (ETR). The oral exposure risk assessment is conducted for granules applied after emergence (broadcast application). Exposure factor and shortcut values are considered in the ETR calculation for different scenarios.

The following tables present the risk assessment for oral exposure resulting from the use of BW01 GB for cereals and oilseed rape. Detailed calculations of the oral risk assessment are provided in the Appendix for each use.

Table 6. Oral risk assessment following granular application for cereals

Crop category	Scenario	BBCH	Honey bee		Bumble bee#		Solitary bee#	
			ETR	Trigger	ETR	Trigger	ETR	Trigger
Cereals	treated crop	< 10	0.00	0.2	0.00	0.036	0.00	0.04
		10 - 29	0.00	0.2	0.01	0.036	0.00	0.04
	weeds	< 10	0.00	0.2	0.04	0.036	0.01	0.04

		10 - 29	0.00	0.2	0.04	0.036	0.01	0.04
	field margin	< 10	0.00	0.2	0.01	0.036	0.00	0.04
		10 - 29	0.00	0.2	0.01	0.036	0.00	0.04
	adjacent crop	< 10	0.00	0.2	0.01	0.036	0.01	0.04
		10 - 29	0.00	0.2	0.01	0.036	0.01	0.04
	next crop	< 10	0.00	0.2	0.02	0.036	0.01	0.04
		10 - 29	0.00	0.2	0.02	0.036	0.01	0.04

ETR: Exposure Toxicity Ratio; BBCH: Growth stage

The honey bee toxicity value divided by a safety factor of 10 was used for these bee species

Bold HQ values identify a potential risk from BW01 GB application

Table 7. Oral risk assessment following granular application for oilseed rape

Crop category	Scenario	BBCH	Honey bee		Bumble bee#		Solitary bee#	
			ETR	Trigger	ETR	Trigger	ETR	Trigger
Oilseed rape	treated crop	< 10	0.00	0.2	0.04	0.036	0.00	0.04
		10 - 29	0.00	0.2	0.04	0.036	0.00	0.04
	weeds	< 10	0.00	0.2	0.01	0.036	0.01	0.04
		10 - 29	0.00	0.2	0.01	0.036	0.01	0.04
	field margin	< 10	0.00	0.2	0.01	0.036	0.00	0.04
		10 - 29	0.00	0.2	0.01	0.036	0.00	0.04
	adjacent crop	< 10	0.00	0.2	0.01	0.036	0.01	0.04
		10 - 29	0.00	0.2	0.01	0.036	0.01	0.04
	next crop	< 10	0.00	0.2	0.02	0.036	0.01	0.04
		10 - 29	0.00	0.2	0.02	0.036	0.01	0.04

ETR: Exposure Toxicity Ratio; BBCH: Growth stage

The honey bee toxicity value divided by a safety factor of 10 was used for these bee species

Bold HQ values identify a potential risk from BW01 GB application

For cereals and oilseed rape, the risk was predicted to be negligible for honeybees (i.e. ETR = 0). The majority of ETR values calculated for bumble bees and all ETRs for solitary bees were below their respective trigger values indicating an acceptable oral exposure to these bee species from the uses of BW01 GB. Only the bumble bee ETR values for weeds at BBCH < 29 and the treated oilseed rape at BBCH 10-29 scenarios were slightly greater than the trigger value of 0.036. However, the risk to bumble bees for these exposure scenarios can be considered acceptable as the calculated ETR values were close to the trigger value and very conservative assumptions were used in the risk assessment for this bee species (please refer to the previous discussion of the contact risk assessment). Results of the oral risk assessment for ornamentals are presented below.

Table 8. Oral risk assessment following granular application for ornamentals

Crop category	Scenario	BBCH	Honey bee		Bumble bee#		Solitary bee#	
			ETR	Trigger	ETR	Trigger	ETR	Trigger
Leafy vegetables	treated crop	< 10	0.01	0.2	0.14	0.036	0.07	0.04
		10 - 49	0.03	0.2	0.50	0.036	0.26	0.04
		50 - 69	0.03	0.2	0.50	0.036	0.26	0.04
		≥ 70	0.00	0.2	0.00	0.036	0.00	0.04
	weeds	< 10	0.02	0.2	0.29	0.036	0.10	0.04
		10 - 49	0.02	0.2	0.29	0.036	0.10	0.04
		50 - 69	0.00	0.2	0.09	0.036	0.03	0.04
		≥ 70	0.00	0.2	0.09	0.036	0.03	0.04
	field margin	< 10	0.01	0.2	0.09	0.036	0.03	0.04
		10 - 49	0.01	0.2	0.09	0.036	0.03	0.04
		50 - 69	0.01	0.2	0.09	0.036	0.03	0.04
		≥ 70	0.01	0.2	0.09	0.036	0.03	0.04
	adjacent crop	< 10	0.01	0.2	0.08	0.036	0.04	0.04
		10 - 49	0.01	0.2	0.08	0.036	0.04	0.04

		50 - 69	0.01	0.2	0.08	0.036	0.04	0.04
		≥ 70	0.01	0.2	0.08	0.036	0.04	0.04
	next crop	< 10	0.01	0.2	0.14	0.036	0.07	0.04
		10 - 49	0.01	0.2	0.14	0.036	0.07	0.04
		50 - 69	0.01	0.2	0.14	0.036	0.07	0.04
		≥ 70	0.01	0.2	0.14	0.036	0.07	0.04

*Leafy vegetables crop category used as surrogate for ornamentals

#The honey bee toxicity value divided by a safety factor of 10 was used for these bee species

ETR: Exposure Toxicity Ratio; BBCH: Growth stage

Bold HQ values identify a potential risk from BW01 GB application

For ornamentals, the ETR values calculated for honey bees were below the trigger value of 0.2 indicating an acceptable risk for this bee species. A potential risk was identified for most of the exposure scenarios for bumble bees and some of the solitary bee scenarios, which can be explained by the very conservative assumptions used in the risk assessment for these bee species.

Acute risk assessment for exposure to contaminated water

The risk assessment for exposure through drinking contaminated water only focuses on honey bees as the high level of water fluxes in this species is considered sufficiently protective for bumble bees and solitary bees (5). The new EFSA guidance recommends using a default value of 11.4 µL/bee in the risk assessment for the water uptake for adult bees (5).

The acute risk assessment from drinking contaminated water for the guttation, surface water and puddle water scenarios are presented below for all the uses of BW01 GB.

Table 9. Risk assessment for exposure to contaminated water

Risk assessment scenario	Water consumption (µL/bee)	ETR	Trigger
Guttation	11.4	0.00	0.2
Surface water	11.4	0.00	0.2
Puddle water	11.4	0.00	0.2

ETR: Exposure Toxicity Ratio

The calculated ETR values were negligible (i.e. ETR = 0.00) for all uses of BW01 GB due to the low water solubility and PECsw values calculated for ferric pyrophosphate (Table 3).

Therefore, the risk to bees for exposure to contaminated water is considerable acceptable, and no further consideration is required.

No studies were submitted to address the larval toxicity and chronic toxicity to adult bees in the assessment report for ferric pyrophosphate (1). Similarly, there is no data available for residues of this active substance in pollen and nectar (1). However, the low water solubility of ferric pyrophosphate (i.e. 0.04 mg/L) together with the estimated strong adsorption to soil and sediment (7) indicate this active substance will be poorly absorbed by plant roots, resulting in negligible or no accumulation of ferric pyrophosphate in nectar and pollen.

BW01 GB pellets are expected to remain on the soil surface until being eaten by the target pests. The remaining pellets may break down releasing ferric pyrophosphate, which may undergo dissociation reactions into iron and phosphate ions in soil. Levels of iron and phosphate are many orders of magnitude higher in nature than those resulting from the application of BW01 GB in the field (7). On this basis, it is unlikely that ferric pyrophosphate

released from the use of BW01 GB in the field will have a negative impact on bees since these non-target organisms are naturally exposed to iron and phosphate ions.

The acute risk to honey bees was demonstrated to be acceptable for all assessed exposure scenarios following application of BW01 GB. This can be explained by the low toxicity of ferric pyrophosphate to this bee species (Table 2). These results are in good agreement with the acceptable risk concluded for other non-target arthropods species from the uses of BW01 GB (4).

Based on the points discussed above, it is not expected that BW01 GB will pose a risk to larvae and to adult bees in the long-term. Therefore, the risk to larvae and the chronic risk to bees from the uses of BW01 GB are deemed to be acceptable.

Conclusions

Although it is considered that BW01 GB will not pose a risk to bees, the acute risk to bees was assessed in this report assuming a worst-case exposure from the uses of BW01 GB. The acute risk assessment was conducted for oral and contact exposures and for the three drinking water scenarios (i.e. guttation, surface water and puddle) in line with the new EFSA guidance. Honey bees, bumble bees and solitary bees were considered in the risk assessment. The toxicity endpoints for honey bees were used as surrogate endpoints in the risk assessment for bumble bees and solitary bees, with an assumption of 10 times greater toxicity.

The risk to honey bees was acceptable for all exposure scenarios considered in the oral and contact risk assessments from all intended uses of BW01 GB, whereas for bumble bees and solitary bees a potential risk was identified for some exposure scenarios indicating the assumptions used in the risk assessment were very conservative for these bee species. The risk assessment for exposure to contaminated water was also acceptable for guttation, surface water and puddle scenarios.

It is not expected that BW01 GB will be toxic to larvae and adults in long-term based on the demonstrated low acute risk to honey bees from the uses of BW01 GB, expected negligible residues of ferric pyrophosphate in nectar and pollen, and the natural occurrence of iron and phosphate in the environment.

1. Draft assessment report for ferric pyrophosphate (2018). Volume 3, Annex B.9: Ecotoxicology.
2. Draft assessment report for ferric pyrophosphate (2018). Volume 3-B.2 (PPP): BW01 GB.
3. EFSA conclusion on the peer review of the pesticide risk assessment of the active substance ferric phosphate (2015). EFSA Journal 2015;13(1):3973.
4. Draft assessment report for BW01 GB (2018). Volume 3, Annex B.9: Ecotoxicology.
5. EFSA guidance document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees) (2013). EFSA Journal 2013;11(7):3295.
6. Guidance document on terrestrial ecotoxicology under council directive 91/414/EEC. (2002). SANCO/10329/2002 rev 2 final, October 2002.
7. Jackson, R. and Healy, S. Environmental behaviour of ferric pyrophosphate in soil and water compartments following broadcast application of the granular bait product BW01 GB in the field (2019). Kerona Scientific Ltd. report.

RMS: In the supplementary assessment of effects on bees the risk to honey bees, bumble bees and solitary bees has been performed according to recommendations given in the EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees) (EFSA Journal 2013;11(7):3295). The assessment indicates that application of a product BW01 GB in line with the critical use

pattern proposed in GAP does not pose the risk due to acute oral and contact exposure to honey bees, bumble bees and solitary bees. The argumentation provided by the applicant for not performing the larval toxicity study and chronic toxicity study in adult bees is considered acceptable. Based on the presented assessment it is concluded that also no unacceptable chronic or reproduction risk for bees is expected taking into account low acute toxicity to bees of ferric pyrophosphate, ubiquitous presence of iron and phosphate in the soil at concentrations much higher than those caused by application of a product BW01 GB and noting the negligible potential absorption of ferric pyrophosphate through roots of plants

B.9.5.1.1 Acute oral toxicity to bees

Based on the data on the active substance presented in point B.9.3.1.1.1, it is predicted that BW01 GB will not demonstrate dietary toxicity to bees. BW01 GB's composition does not include any substances, apart from the active substance, that could significantly increase toxic properties of the formulation (as demonstrated by the acute dietary toxicity study for mammals conducted both for the active substance - B.9.1.2.1 and the formulation B.9.1.2.1, the remaining substances do not affect toxicological properties of the product), therefore ecotoxic properties of the PPP do not differ from the data for the active substance. In addition, the granule study results (see B.2.8.5.2 and B.2.8.5.3) indicate that the product is non-dusty and attrition-resistant, which makes the risk resulting from dust carryover during application negligible.

B.9.5.1.2 Acute contact toxicity to bees

Based on the data on the active substance presented in point B.9.3.1.1.2, it is predicted that BW01 GB will not demonstrate contact toxicity to bees. BW01 GB's composition does not include any substances, apart from the active substance, that could significantly increase toxic properties of the formulation (as demonstrated by the acute dietary toxicity study for mammals conducted both for the active substance - B.9.1.2.1 and the formulation B.9.1.2.1, the remaining substances do not affect toxicological properties of the product), therefore ecotoxic properties of the PPP do not differ from the data for the active substance. In addition, the granule study results (see B.2.8.5.2 and B.2.8.5.3) indicate that the product is non-dusty and attrition-resistant, which makes the risk resulting from dust carryover during application negligible.

B.9.5.1.3 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.9.5.1.4 Effects on honey bee development and other honey bee 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.9.5.1.5 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.9.5.1.6 Cage and tunnel tests

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. Thus, studies in cages and tunnels were not conducted.

B.9.5.1.7 Field tests with honeybees

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. Thus, a field study with the use of honey bees was not conducted.

B.9.5.2. Effects on non-target arthropods other than bees

B.9.5.2.1. Standard laboratory testing for non-target arthropods

A study of the effect on *Aphidius rhopalosiphi* and *Typhlodromus pyri* 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 *Poecilus cupreus* and *Aleochara bilineata* was conducted.

Laboratory studies

B.9.5.2.1/01 Lethal and sublethal effects of molluscicide BW01 GB on beetle *Poecilus cupreus*

Report: J. Winkler 2014, Lethal and sublethal effects of molluscicide BW01 GB on beetle *Poecilus cupreus*
Study code: 0001/0100/E
Guideline: IOBC, BART, EPPO [Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. M.P. Candolfi, S. Blümel, R. Forster et al. (2000)]
GLP: yes

Executive Summary:

The study of the lethal and sub-lethal effects of the test item, molluscicide BW01 GB for beetles *Poecilus cupreus* was conducted. The aim of the study was to compare the survival and amount of eaten food by beetles in relation to the control and after the exposure to the test item, in order to determine LDx, EDx and NOED/LOED values for the endpoints. Tested doses:

- preliminary test: control (0 g/m^2); 0,4 g/m^2; 0,6 g/m^2; 2,5 g/m^2; 5,0 g/m^2; 10,0 g/m^2
- definitive test: control (0 g/m^2); 0,4 g/m^2; 2,5 g/m^2; 5,0 g/m^2; 10,0 g/m^2; 23,0 g/m^2

During the current study and performed statistical calculations it was indicated, that test item - BW01 GB, has no impact on survival of beetles *Poecilus cupreus* nor the amount of pupae eaten by them. BW01 GB demonstrates no toxic effects on beetles in relation to those parameters in the tested range of dosed, also the highest one – 23

g/m^2. Calculation of values EDx for study endpoints, was not possible due to mathematical reasons. Final results are presented in the table CP 10.3-2

Table 9.5-1 Final results for lethal and sublethal effects of molluscicide BW01 GB on beetle *Poecilus cupreus*

ED_{10} mortality	n/d*
ED_{20} mortality	n/d*
ED_{50} mortality	n/d*
NOED mortality	n/d* (≥ 23 g/m^2)**
LOED mortality	n/d* (≥ 23 g/m^2)**
ED_{10} amount of eaten food	n/d*
ED_{20} amount of eaten food	n/d*
ED_{50} amount of eaten food	n/d*
NOED amount of eaten food	n/d* (≥ 23 g/m^2)**
LOED amount of eaten food	n/d* (≥ 23 g/m^2)**

*n/d – impossible to determine due to mathematical reasons and with a use of mathematical software programs
**results provided basing on a real observations

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BW01 GB

Description: turquoise granulate

Batch: 032014-P82

Content of active ingredient: 3 % of iron pyrophosphate

Storage: dry and cool place

2. Vehicle and/or positive control: dimethoate [redacted]

3. Test animals

Species: beetle *Poecilus cupreus*

Age: 2-10 weeks from reaching the imago stage

Weight: n.a.

Source: BIO-TEST LABOR GMBH SAGERHEIDE

Diet: pupae of *Musca domestica* housefly ad libitum, 2-5 times a week

Acclimation period: 7 days

Environmental conditions

Temperature: 20ºC ± 2ºC

Humidity: 60-90%

Photoperiod: continuous fluorescent light of intensity 200-2000, 16h light: 8h darkness

B. STUDY DESIGN AND METHODS:

1. In life dates: preliminary test 4 - 18.08.2014, definitive test 8 - 22.09.2014

2. Experimental treatments

Preliminary test

In order to determine the appropriate doses for the definitive test, preliminary test was conducted with wide range of test item doses. In the study, a control and following, five doses of the test material were used: 0,4 g/m^2, 0,6 g/m^2, 2,5 g/m^2, 5 g/m^2 and 10 g/m^2 (respectively 4000 g/ha, 6000 g/ha, 25000 g/ha, 50000 g/ha and 100000 g/ha). Doses were determined basing on the area unit. 7 days before the test item application, beetles were places in test containers and fed with thawed, cut in half houseflies pupae. Then, containers with beetles were placed in test conditions. Three days before the test item application feeding was suspended and uneaten ford removed. On the begging of experiment day, beetles were transferred to the sand surface (if they were burrowed) and sand surface was leveled. The test was conducted for 14 days. Test conditions and feeding frequency were the same as in the definitive study. 5 replicates per each dose and control, with 3 males and 3 females introduced into each replicate were used.

Definitive test

In the definitive study, a control and five doses of the test material were used. Each replicate consistent of 5 replicates with 3 males and 3 females introduced into each replicate. The following test item doses were used: 0,4 g/m^2; 2,5 g/m^2; 5 g/m^2; 10 g/m^2 and 23 g/m^2 (respectively 4000 g/ha, 25000 g/ha, 50000 g/ha, 100000 g/ha and 230000 g/ha). Exposure time was 2 weeks.

Reference test

In the study [redacted](dimethoate [redacted] was used as a reference item, where dimethoate at dose 400g/ha is the active substance. The validation study involved testing a wide range of doses of test reference item: 0,1 g/ha; 1 g/ha; 10 g/ha; 100 g/ha; 1000 g/ha. Validation was performed in parallel with the preliminary test in the same conditions.

3. Observations

Preliminary test

On days 0, 1, 2, 4, 7, 10/11 and 14 mortality and Mount of eaten pupae, as well as behavior changes were recorded. During the experiment the environmental conditions were measured: light intensity – everyday, as well as temperature and air relative humidity were continuously recorded by temperature and humidity recorder.

Definitive test

During the experiment, the following observations and measurements were performed:

• after 1 - 3 hours, and additionally after further 2 hours in case of occurring intoxication effects, as well as on days 1, 2, 4, 7, 10/11, 14 mortality and un abnormal behaviour;

• mortality – beetles were considered dead if there was no movement of legs and antennae, and if the insects did not respond to gentle mechanical stimulation. Alive beetles, but which were unable to move were marked as "drastically damaged" and moved to the edge of the test vessel. During the following observations it was verified if they regained the ability to move (not longer than after 18 hours). If at that time they did not change position, then were considered dead;

• food intake – recorded on days 2, 4, 7, 10/11, as the number of eaten pupae (partially or totally) per living beetle for each feeding, each week and each tested dose as well as the whole experience;

• light intensity
• weight of test containers
• temperature and the humidity

4. Statistical calculations

The mortality observation results were analyzed statistically to determine the endpoints values of the experiment. For statistical analysis of beetles mortality ToxRat Professional program was used, while for the amount of eaten by beetles pupae, Statistica program was used.

Raw data were copied to the Excel software and then edited for Statistica program Leeds. Data were copied to the Statistica software and calculations were performed. Data analysis begun with the calculation of the basic parameters of the distribution for the number of eaten pupae. Results were analyzed for their statistical parameters (mean, median, standard deviation mode), and their diversity and number of values were determined. The difference between the medians and the indicated the symmetry of the distribution or its lack.

In the next stage, normality of the distribution was tested. Graph test and histogram (deviation from a Gaussian curve) of distribution showed the normality or lack of normality properties. If the coefficient of variation deviated from the range of <20% - 25%>, it additionally confirmed that the distribution is not normal.

Distribution characteristics forced choice as analysis tool as a non-parametric test - Mann-Whitney test. The hypothesis was verified at the significance level ($p < 0,05$). As a measure a median central tendency was adopted.

The reduction percentage was calculated by subtraction from the control value, the value of the concentration (dose), both from the same time point. Then it was calculated what percentage is the result and it was multiplied by 100%. The probit regression calculations were performed. The connection between test item dose and number of eaten pupae were used to calculate ED_x. Hypotheses in the current analysis were tested at significance level ($p < 0,05$).

5. Deviations from the Guideline/Study Plan

Due to lack of dedicated calculation sheet of ToxRat Professional for the current study Guideline, the calculations of the beetles' mortality were performed using a modified sheet designed for the study, where similar parameters are observed at similar time intervals. During the analysis program did not detect any errors, what guarantees accuracy of the calculation.

Due to lack of dedicated calculation sheet of ToxRat Professional for the current study Guideline, the calculations of the amount of eaten by beetles pupae were performed using a modified sheet as well as Statistica program.

The Study Plan assumed that on the basis of results of the validation study, a single dose would be used in the definitive study. Selected dose would correspond to the LD_{50} for the mortality of beetles. In relation to the requirements of the Guideline, which states that mortality in reference item test should be between 65 – 100%, during the experiment mortality value LD_{65} was taken into consideration.

Guideline suggest to evenly wet sand in the test vessels just before placing beetles in them. During the current study, sand was wetted 3 days prior to placing beetles in the test vessels. Such method of sand preparation allowed to obtained uniformly wetted sand in it whole volume. It is assumed that this deviation did not affect the study performance and the final results.

According to the Gudeline, a reference substance should be Afugan, but due to the fact that it's unavailable for sane, the difference reference item was used – [redacted], where dimethoate is the active substance. Dimethoate is the reference substance suggested for use in the other tests on arthropods not being the pests, for example bees

(OECD 213 and OECD 214). In the current study, reference test with substance [redacted] was validated and it was proved that it can be used as a reference item in the test performed for beetles *Poecilus cupreus.*

II. RESULTS AND DISCUSSION

A. FINDINGS

Table 9.5-2 Final results of the preliminary study

Dose [g/m^2]	**control**	**0,4**	**0,6**	**2,5**	**5,0**	**10,0**
Number of introduced insects	30	30	30	30	30	30
Number of survived insects after 14 days of experiment	30	30	30	30	30	30
Mortality [%]	0	0	0	0	0	0
NOEC mortality	n/d** (≥ 10 g/m^2)***					
LOEC mortality	n/d** (≥ 10 g/m^2)***					
Total number of provided pupae *	180	180	180	180	180	180
Total number of eaten pupae	139,5	140,5	134,5	135	132	138
% of eaten pupae during the first week of the experiment	70,83	72,08	67,08	67,08	62,92	66,25
% of eaten pupae during the second week of the experiment	90,83	90,00	90,00	90,83	94,17	97,50
Total % of eaten pupae	77,50	78,06	74,72	75,00	73,33	76,67
% Reduction	not applicable	not applicable	3,3	2,5	4,2	0,8
Statistical significance (YES/NO)	not applicable	not applicable	no	no	no	no
EC10 amount of eaten food	n/d**					
EC20 amount of eaten food	n/d**					
EC50 amount of eaten food	n/d**					
NOEC amount of eaten food	n/d** (≥ 10 g/m^2)***					
LOEC amount of eaten food	n/d** (≥ 10 g/m^2)***					

*during the first week of the experiment 120 were introduced and during the second week, 60 were introduced
**n/d –impossible to determine due to mathematical reasons
*** results provided basing on real observations

Table 9.5-3 Final results - definitive test

Dose [g/m^2]	**control**	**0,4**	**2,5**	**5,0**	**10,0**	**23,0**
Number of introduced insects	30	30	30	30	30	30
Number of survived insects after 14 days of experiment	30	30	30	30	30	30
Mortality [%]	0	0	0	0	0	0

NOEC mortality	n/d** (≥ 23 g/m^2)***					
LOEC mortality	n/d** (≥ 23 g/m^2)***					
Total number of provided pupae *	180	180	180	180	180	180
Total number of eaten pupae	161	164	162,5	161	168,5	164
% of eaten pupae during the first week of the experiment	87,50	90,00	88,75	87,08	92,50	89,58
% of eaten pupae during the second week of the experiment	93,33	93,33	93,33	94,17	95,83	94,17
Total % of eaten pupae	89,44	91,11	90,28	89,44	93,61	91,11
% Reduction	not applicable	n/d**	n/d**	n/d**	n/d**	n/d**
Statistical significance (YES/NO)	not applicable	n/d**	n/d**	n/d**	n/d**	n/d**
EC10 amount of eaten food	n/d**					
EC20 amount of eaten food	n/d**					
EC50 amount of eaten food	n/d**					
NOEC amount of eaten food	n/d** (≥ 23 g/m^2)***					
LOEC amount of eaten food	n/d** (≥ 23 g/m^2)***					

*During the first week of the experiment 120 were introduced and during the second week, 60 were introduced
** n/d* - values not determined due to mathematical reasons by statistical software
*** results provided basing on a real observations

B. OBSERVATIONS

During the preliminary study there was no beetles' mortality observed. Also no statistically significant inhibition of the amount of eaten pupae in relation to the control for the final results of the experiment were observed.

During the study there were no beetles' mortality nor intoxication symptoms observed. Also no statistically significant inhibition of the amount of eaten pupae in relation to the control for the final results of the experiment were observed.

During the current reference test, 100% mortality of beetles as well as 100% inhibition of the amount of eaten food were observed at an active substance dose of 350 g/ha. Reference test has met the validity criteria of Guideline stating that in the reference test, beetles mortality should be in the range 65 ± 35%.

III. CONCLUSIONS

During the current study and performed statistical calculations it was indicated, that test item - molluscicide BW01 GB, has no impact on survival of beetles *Poecilus cupreus* nor the amount of pupae eaten by them. Molluscicide BW01 GB demonstrates no toxic effects on beetles in relation to those parameters in the tested range of dosed, also the highest one – 23 g/m^2 Calculation of values ED_x for study endpoints, was not possible due to mathematical reasons.

RMS comments:
The study of lethal and sublethal effects of molluscicide BW01 GB on beetle *Poecilus cupreus* was performed in GLP conditions and according internationally recognised guidelines. 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 beetles or toxic symptoms were observed within 14 days of exposure at a dose of 4 kg/ha, 25 kg/ha, 50 kg/ha, 100 kg/ha and 230 kg/ha. The study is considered acceptable with following endpoint:
14 day NO EFFECT APPLICATION RATE $(NoEAR)_{mortality/food\ consumption}$ for beetle Poecilus cupreus ≥ 230 kg of product/ha

B.9.5.2.1/02 Lethal and sublethal effects of molluscicide BW01 GB on Aleochara bilineata Gyllenhal (Coleoptera: Staphylinidae)

Report: Pecorari F. 2015 Evaluation of the chronic effects of the product BW01 GB on the rove beetle *Aleochara bilineat*a Gyllenhal under Laboratory Conditions

Study code: BT116/15

Guideline: ESCORT I Guidance Document (Barret et al., eds. 1994) and the ESCORT II Guidance Document (Candolfi et al., eds. 2001). The study was carried out following IOBC/WPRS guidelines (Candolfi M.P. et al., 2000).

GLP: yes

Executive Summary:

The effects of the test item BW01 GB on the reproduction of rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) were tested with a Laboratory Study, according to GLP regulations.

The results obtained in the trial showed that the test item BW01 GB did not cause significant mortality on the test system Aleochara bilineta Gyll. (Coleoptera: Staphylinidae) at the tested application rates.

The LR 50 value after 4 weeks of exposure is greater than 230.00 kg test item/ha (equivalent to 6.90 kg of a.s./ha).

Regarding the reproduction capacity, the test item at T5 application rate caused significant reduction of the mean number of offspring produced per female, compared to the number of offspring produced per female in the control group. This reduction was 38.27 %, so the ER 50 value is greater than 230.00 kg of test item/ha (equivalent to 6.90 kg of a.s./ha). The NOEC value is 100.00 kg test item/ha (equivalent to 3.00 kg of a.s./ha).

No behavioural abnormalities of treated adults were assessed at the end of exposure phase.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: BW01 GB

Description: Molluscicide

Batch: 032014-P82

Content of active ingredient: Ferric Pyrophosphate: 3.0 %

2. Reference item: dimethoate

3. Test animals

Species: *Aleochara bilineata* Gyll.

Age: Adults (3 days after hatching from Delia antiqua pupae)

Source: De Groene Vlieg (Netherlands)

Diet: *Tenebrio molitor* (frozen larvae), Tap water provided in order to restore the soil humidity

Acclimation period: not relevant

Environmental conditions

Temperature: 18.33 - 21.67 °C

Humidity: 64,50 - 85,00 %

Photoperiod: 325 - 900 lux, 16 h light and 8 h darkness

B. STUDY DESIGN AND METHODS:

1. Experimental period: 23rd July 2015 - 09th October 2015

2. Experimental treatments:

The test consisted of an exposure and a hatching phase. For the exposure phase, four replicates were set up for each treatment group. Each replicates included 10 male and 10 female beetles.

All onion fly pupae recovered from one exposure test unit were placed in to one hatching test unit, so that for the hatching phase there were four replicates for each treatment group too.

The experimental phase consisted of 7 treatment groups:

- Test item at 5 application rates
- Control group
- Reference item group

At the start of the exposure phase, ten pairs of male and female adult beetles were exposed to treated substrate for 28 days. The beetles were placed on the treated substrate of each replicate after the test item application.

The beetles were fed approximately one hour after their introduction and then every 1 to 3 days depending on the food consumption. Food was placed on the surface of the substrate.

After 7, 14 and 21 days, approximately 500 onion fly pupae were added and carefully mixed with the substrate of each test unit and completely covered with substrate.

The number of pupae was determined by weight on each occasion. During the entire test, an amount of approximately 1500 onion fly pupae were used per test unit.

Four weeks after test initiation, the exposure phase was finished and the adult beetles were removed from the substrate.

At the end of the exposure phase, the number of dead and alive beetles was recorded. However, these data were not necessary to evaluate the test. The substrate and the parasitized onion fly pupae were returned to the climatic chamber in the original test units without the lid in order to allow the drying of the substrate for one week.

After this week, the pupae were separated from the substrate with a sieve and placed in the hatching test units.

Emergence of *A. bilineata* beetles was monitored until the control treatment fell below a rate of two beetles per replicate per day.

3. Application rates

The experimental phase was organized as described in the following table:

Table 9.5-4 Application rates

Treatments	kg test item/ha	kg a.s./ha	g test item/m²	N° adults/ cage	N° replicates	Identification Code
Control	0.00	0.00	0.00	10♀+10♂	4	CTRL a ÷ CTRL d
Test item - T1	4.00	0.12	0.40	10♀+10♂	4	T1 a ÷ T1 d
Test item - T2	25.00	0.75	2.50	10♀+10♂	4	T2 a ÷ T2 d
Test item - T3	50.00	1.50	5.00	10♀+10♂	4	T3 a ÷ T3 d
Test item - T4	100.00	3.00	10.00	10♀+10♂	4	T4 a ÷ T4 d
Test item - T5	230.00 [1]	6.90	23.00	10♀+10♂	4	T5 a ÷ T5 d
Reference item	3.00 [2]	0.537 [2]	0.30	10♀+10♂	4	R a ÷ R d

[1] Maximum field application rate: 50 kg/ha x MAF (soil substrate)
[2] This concentration is chosen in line with the IOBC Guideline and it is expected to cause a reduction of reproductive capacity ≥50 %.
Number of applications/year: 6
MAF (soil substrate): 4.6

4. Observations

The adult beetles emerging from the fly pupae were counted in each replicate until no more than two beetles per day emerged in the control group. Behavioural and survival of the test system were assessed at the end of the exposure phase.

5. Statistical calculations

The software ToxRat Pro Version 3.1.0 was used to perform the statistical evaluation about significance on reproduction output.

6. Deviations from the Guideline

None.

II. RESULTS

Mortality:

Table 9.5-5 Mortality after 4 weeks of exposure

Treatment	kg test item/ha	kg a.s./ha	g test item/m²	% Mean Mortality	% Mean Corrected Mortality
Control	0.00	0.00	0.00	2.50	===
Test item T1	4.00	0.12	0.40	2.50	0.00
Test item T2	25.00	0.75	2.50	5.00	2.56
Test item T3	50.00	1.50	5.00	5.00	2.56
Test item T4	100.00	3.00	10.00	7.50	5.13
Test item T5	230.00	6.90	23.00	2.50	0.00
Reference item	3.00	0.537	0.30	12.50	10.26

The results showed no significant mortality of the test system when treated by the test item.

The LR_{50} of the test item BW01 GB, evaluated after 4 weeks of exposure, was greater than the maximum application rate tested, equivalent to 230.00 kg test item/ha (corresponding to 6.90 kg of a.s./ha).

Reproduction:

Table 9.5-6 Reproduction performance of the exposed beetles

Treatment	kg test item/ha	kg a.s./ha	g test item/m²	Mean number of offspring/female	SD	% Fertility [1]	% R	Sig. *
Control	0.00	0.00	0.00	86.35	4.93	57.57	===	
Test item - T1	4.00	0.12	0.40	86.05	4.49	57.37	0.35	-
Test item - T2	25.00	0.75	2.50	86.85	2.06	57.90	-0.58	-
Test item - T3	50.00	1.50	5.00	83.98	5.95	55.98	2.75	-
Test item - T4	100.00	3.00	10.00	84.58	3.79	56.38	2.06	-
Test item - T5	230.00	6.90	23.00	53.30	0.72	35.53	38.27	+
Reference item	3.00	0.537	0.30	39.10	2.92	26.07	54.72	

1 = Total number of offspring/6000*100 where 6000= total number of pupae introduced
% R = % reduction in reproduction
* + : significant - : non-significant for α=0.05 (William's Multiple t-test procedure, ToxRat Pro 3.1.0)

The results of the reproduction phase showed a statistically significant reduction in the mean number of the offspring produced by the treated beetles with T5 application rate, respect to the mean number of the offspring produced by the beetles of the control group.

Based on these results the ER_{50} was greater than the maximum application rate tested, equivalent to 230.00 kg test item/ha (corresponding to 6.90 kg of a.s./ha).

III. CONCLUSIONS

The results obtained in the trial showed that the test item BW01 GB did not cause significant mortality on the test system *Aleochara bilineta* Gyll. (Coleoptera: Staphylinidae) at the tested application rates.

The LR_{50} value after 4 weeks of exposure is greater than 230.00 kg test item/ha (equivalent to 6.90 kg of a.s./ha).

Regarding the reproduction capacity, the test item at T5 application rate caused significant reduction of the mean number of offspring produced per female, compared to the number of offspring produced per female in the control group. This reduction was 38.27 %, so the ER_{50} value is greater than 230.00 kg of test item/ha (equivalent to 6.90 kg of a.s./ha). The NOEC value is 100.00 kg test item/ha (equivalent to 3.00 kg of a.s./ha).

RMS comments:
The study of effects of molluscicide BW01 GB on the reproduction of rove beetle *Aleochara bilineta Gyllenhal* was performed in GLP conditions and according internationally recognised guidelines. 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. No significant mortality of beetles was observed within 4 weeks of exposure at a dose of 4 kg/ha, 25 kg/ha, 50 kg/ha, 100 kg/ha and 230 kg/ha, however at an application rate of 230 kg/ha there was 38.27% reduction of number of offspring produced per female. The study is considered acceptable with following endpoint:
28 day NO EFFECT APPLICATION RATE $(NoEAR)_{mortality}$ for beetle *Aleochara bilineta Gyllenhal* ≥ 230 kg product/ha
EFFECTIVE APPLICATION $RATE_{50\ fertility}$ > 230 kg product/ha (6.9 kg of a.s./ha)
NO EFFECTS APPLICATION $RATE(NoEAR)_{fertility}$ - 100 kg of product/ha (3.0 kg of a.s./ha)

B.9.5.3 Extended laboratory testing, aged residue studies with non-target arthropods

Since no effects were observed following the standard laboratory studies described in point B.9.5.2.1 and the risk factor analysis does not indicate a risk for the non-target arthropod species investigated, extended laboratory studies and residue studies with the use of non-target arthropods are not required.

B.9.5.4 Semi-field studies with non-target arthropods

Since no effects were observed following the standard laboratory studies described in point B.9.5.2.1 and the risk factor analysis does not indicate a risk for the non-target arthropod species investigated, semi-field studies with the use of non-target arthropods are not required.

B.9.5.5 Field studies with non-target arthropods

Since no effects were observed following the standard laboratory studies described in point B.9.5.2.1 and the risk factor analysis does not indicate a risk for the non-target arthropod species investigated, field studies with the use of non-target arthropods are not required.

B.9.5.6 Other routes of exposure for non-target arthropods

For the BW01 GB plant protection product, there is no other exposure route than through contact, thus a study of other exposure routes for non-target arthropods is not required.

B.9.6 Risk Assessment for Arthropods

B.9.6.1 Risk for Honeybees

Hazard quotient for oral or contact exposure of honey bees in accordance with the EPPO (2010) Environmental risk assessment scheme for plant protection products (Chapter 10: Honeybees (PP 3/10(3)) Bulletin OEPP/EPPO Bulletin 40, 323-331 has been calculated with the use of the following formula:
hazard quotient = maximum application dose [g of the active substance/hectare] / LD_{50} [µg of the active substance/bee]

Table 9.3-1 Hazard quotient for oral or contact exposure of honey bees

Substance	Exposure route	LD_{50}	Max. single application rate	Hazard quotient (HQ)	HQ assessment trigger
Ferric pyrophosphate	oral	> 100 µg as/bee	1500 g/ha	15	50
	contact	> 100 µg as/bee		15	50

Based on the laboratory study results, ferric pyrophosphate is considered non-toxic to bees. The calculated values of hazard quotient for oral and contact exposure are much lower than the trigger value 50, which indicates that the intended use of ferric pyrophosphate does not pose a risk to bees. Ferric pyrophosphate will not negatively affect bees and bee colonies if it is used in accordance with the rules of good agricultural practice and label instructions. Moreover, the BW01 GB plant protection product has a form of non-dusty and attrition-resistant granules, which makes the risk resulting from dust carryover during application negligible.

RMS comments:

The assessment of risk to honeybees has been performed in compliance with EPPO Standards. The hazard quotients for acute oral and contact exposure are below 50, the Commission Regulation (EU) No. 284/2013 criterion, indicating low acute risk to honey bees. The applicant did not submit any additional studies to address the chronic toxicity to adult worker bees and the toxicity to larvae, which is required by the new EFSA Guidance on risk assessment on bees (EFSA Journal 2013;11(7):3295). However, data on chronic toxicity and reproductive toxicity of an active substance, iron pyrophosphate, and of the product BW01 GB for four species of other non-target arthropods living in a soil have demonstrated very low risk to arthropods due to chronic exposure, therefore it might be reasonably expected that application of this product in compliance with label instructions will not pose an unacceptable risk to adult worker bees, bumble bees or solitary bees, and to larvae.

B.9.6.2 Risk for Non-target arthropods other than bees

BW01 GB is a granular bait used from 1 to 6 times per season in a maximum dose of 50 kg/ha (=1500 g of the active substance/ha). The product is used to control slugs and snails in edible and inedible plant cultivations. Since the granules are scattered directly to soil surface, the potential exposure to off-field non-target arthropods is minimum. Hazard quotient calculations according to ESCORT 2 guidelines are inappropriate for granular plant protection products. In the case of granules, it is recommended that risk assessment be conducted in accordance with ESCORT 1 guidelines, namely studies of the formulation should be conducted with the use of soil-dwelling species. Therefore, risk assessment for non-target arthropods other than bees is based on a toxicological study for *Poecilius cupreus* and *Aleochara bilineata*. Study on the effect of BW01 GB on *Poecillus cupreus* beetle showed that exposure of the organisms studied to the concentration of 6900 g of the active substance per hectare (that is 4.6 times higher than the suggested maximum application dose) produced an effect much lower that the trigger value 30% in sub-lethal effects, defined in the ESCORT 1 guideline. In the study on the effect of BW01 GB on *Aleochara bilineata* beetle, a dose equalling a single and double application of BW01 GB produced an effect much lower that the trigger value 30%. Even though the effect for a dose equalling a sixfold application slightly exceeds the trigger value, it needs to be noted that when the product is used in accordance with the rules of good agricultural

practice and label instructions, it will be eaten by slugs/snails, thus its concentration in soil will be much lower than calculated for the worst-case scenario concentration corresponding to sixfold cumulative dose. Thus, the risk to non-target arthropods other than bees, resulting from the use of BW01 GB in accordance with the rules of good agricultural practice and label instructions, is acceptable.

Due to the negligible exposure to the formulation BW01 GB in off-field areas the risk to off-field non-target arthropods is considered acceptable. No further data or risk assessment is required.

RMS comments:

A risk for non-target arthropods other than bees is considered as low. This conclusion is supported by results of two studies of toxicity of the product BW01 GB for non-target arthropods other than bees: Aleochara bilineata Gyllenhal and Poecilus cupreus.. The studies provided the following toxicity endpoints:

for beetle Aleochara bilineata Gyllenhal:

28 day NO EFFECTS APPLICATION RATE $_{mortality}$ > 230 kg product/ha

EFFECTIVE APPLICATION RATE$_{50\ fertility}$ > 230 kg product/ha (6.9 kg of a.s./ha)

NO EFFECTS APPLICATION RATE (NoEAR)$_{fertility}$ - 100 kg of product/ha (3.0 kg of a.s./ha)

for beetle Poecilus cupreus

14 day NO EFFECTS APPLICATION RATE$_{mortality/food\ consumption}$ for beetle Poecilus cupreus > 230 kg/ha

The value of lowest NO EFFECTS APPLICATION RATE is at least 2 times higher and values of other endpoints more than 4 times higher, than the maximum application rate of the product 50 kg/ha (1.5 kg a.s./ha) as recommended in the label. Therefore the conclusions based on active substance data that a risk for in-field non-target arthropods is low may be confirmed by product data. For the non-target arthropods dwelling in off-field areas the risk will be even lower due to lower than in in-field areas level of exposure.

B.9.7 Effects on Non-target Soil Meso- and Macrofauna

B.9.7.1 Earthworms

B.9.7.1.1 Earthworms – sub-lethal effects

Studies for the formulation are not required because the data for the active substance is treated as the worst-case scenario and there is no evidence that would suggest that the formulation is more toxic than the active substance. Based on the data on the active substance (see B.9.4.1), it might be predicted that the BW01 GB plant protection product will not demonstrate sub-lethal toxicity to earthworms.

B.9.7.1.2 Earthworms – field studies

Relevant analysis of the risk factor does not indicate that there exists a long-term risk to earthworms, thus a field study is not necessary.

B.9.7.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

B.9.7.2.1 Species level testing

Studies for the formulation are not required because the data for the active substance are treated as the worst-case scenario and there is no evidence that would suggest that the formulation is more toxic than the active substance. Based on the data on the active substance (see B.9.4.2), it might be predicted that the BW01 GB plant protection product will not demonstrate toxicity to soil-dwelling species such as *Folsomia candida* and *Hypoaspis aculeifer*.

B.9.7.2.2 Higher tier testing

Laboratory studies did not demonstrate any significant impact of ferric pyrophosphate on the soil-dwelling species such as *Folsomia candida* and *Hypoaspis aculeifer*. Risk assessment for these species does not indicate that there is a risk, thus higher-tier studies are considered unnecessary.

B.9.8 Risk Assessment for Non-target Soil Meso- and Macrofauna

The value of the predicted environmental concentration to perform an acute risk assessment for BW01 GB was calculated based on a single maximum dose of 50 kg of the product/ha. Assuming soil depth of 5 cm and soil density of 1.5 g/cm^3, application dose is 66,67 mg of the product/kg of dry weight of soil, giving a maximum single application dose of 1500 g of the active substance/ha. Short-term and long-term actual concentrations and time weighted average concentrations for the active substance were not calculated because reliable DT_{50} value in soil was not obtained for ferric pyrophosphate. Therefore, assuming multiple application in a season as the worst-case scenario, it is assumed that the entire BW01 GB is applied in one total dose. Table B.9.8.1-1 presents the obtained maximum predicted environmental concentrations for a single application and 6 applications of BW01 GB. Since iron and phosphorus naturally occur in soil, the table also contains comparison of natural concentrations of these elements in soil.

Table 9.8.1 -1 Predicted environmental concentration in soil (mg/kg).

	Formulation	$Fe_4(P_2O_7)_3$		Fe^{3+}*		$P_2O_7^{4-}$**	
Soil depth	5 cm	5 cm	1 cm	5 cm	1 cm	5 cm	1 cm
PEC (1 application)	66,67	2	10	0,6	3	1,4	7
PEC (6 applications)	400,02	12	60	3,6	18	8,4	42
Natural iron and phosphorus, expressed as pyrophosphate, concentration in soil	-	-		10 000 - 50 000		275 - 16 500	
Ratio between the natural concentration and PEC - 1 application	-	-		~ 16 667 – 83 333		~ 196 – 11 786	
Ratio between the natural concentration and PEC - 6 applications	-	-		~ 2 778 – 13 889		~ 33 – 1 964	

* 30 % of ferric pyrophosphate

** phosphorus expressed as $P_2O_7^{4-}$ - 70 % of ferric pyrophosphate

However, it is reasonable to state that the assumption of one total application does not reflect the conditions of product use. When assessing exposure that includes the worst-case scenario for multiple application, the following aspects should be taken into account:

- additional applications make sense only when the product was ingested by slugs/snails but the their presence is still observed
- ferric pyrophosphate and iron and phosphorus ions disperse in the environment via degradation. However, the concentration of iron and phosphorus in soil decreases significantly as a result of absorption by plants because they use these elements as nutrients. This decrease is usually replenished by fertilization. The use of iron and phosphorus in fertilizers has been described in detail in chapter B.8.1.

B.9.8.1 Risk assessment for earthworms

To assess the risk to earthworms resulting from the use of the BW01 GB product, the ratio between long-term toxicity and exposure was calculated using initial predicted concentrations in soil after a single and multiple application of the product. The results have been presented in table B.9.8.1 -2 .

Table 9.8.1-2 TER value for BW01 GB

Study	PEC [mg s.a./kg]	NOEC	TER	Trigger value	Reference
Earthworms – sub-lethal effects	1 application – 2 mg/kg	≥ 1000 mg as/kg of soil	≥ 500	> 5	Table B.8.1-1. Predicted environmental concentration in soil (mg/kg).
	6 applications – 12 mg/kg		≥ 83		

The obtained values of the ratio between chronic toxicity and exposure are above the trigger value for both single and sixfold application of BW01 GB, thus the risk to earthworms resulting from the use of PPP containing ferric pyrophosphate in accordance with the rules of good agricultural practice and label instructions is acceptable.

B.9.8.2 Risk assessment for Non-target Soil Meso- and Macrofauna (other than earthworms)

To assess the risk to soil-dwelling species such as *Folsomia candida* and *Hypoaspis aculeifer* resulting from the use of the BW01 GB product, the ratio between long-term toxicity and exposure was calculated using initial predicted concentrations in soil after a single and multiple application of the product. The results have been presented in table B.9.8.2 -1 .

Table 9.8.2-1 TER value for BW01 GB

Study	PEC [mg s.a./kg]	NOEC	TER	Trigger value	Reference
Folsomia candida	1 application – 2 mg/kg	≥ 1000 mg as/kg of soil	≥ 500	> 5	Table B.8.1-1. Predicted environmental concentration in soil (mg/kg).
	6 applications – 12 mg/kg		≥ 83		

Hypoaspis aculeifer	1 application – 2 mg/kg	1000 mg as/kg of soil	500	> 5	Table B.8.1-1. Predicted environmental concentration in soil (mg/kg).
	6 applications – 12 mg/kg		83		

The obtained values of the ratio between chronic toxicity and exposure are above the trigger value for both single and sixfold application of BW01 GB, thus the risk to -dwelling species such as *Folsomia candida* and *Hypoaspis aculeifer* resulting from the use of PPP containing ferric pyrophosphate in accordance with the rules of good agricultural practice and label instructions is acceptable.

RMS comments:

The potential long-term risk to earthworms resulting from exposure to iron pyrophosphate was assessed, was based on active substance toxicity data (B.9.7.1/01), by comparing the worst case $PEC_{soil,}$ values after single application and after 6 applications of product (B.8.1-1) with respective NOEC values ((B.9.7.1/01). The resulting TER values are well above a trigger value of 5 indicating a low long-term risk to earthworms after application of product BW01 GB in line with recommended application rates.

The effects of active substance, iron pyrophosphate, on mortality and reproduction of non-target arthropods were assessed additionally based on effects on two species dwelling in soil: Folsomia candida and Hypoaspis aculeifer (see point B.9.8) taking into account that product BW01 GB is applied directly on the soil as granules either manually or by means of an applicator the applicant. For Folsomia candida the $NOEC_{mortality/reproduction}$ was found to be greater than or equal to 1000 mg test item/kg soil dry weight, and for the predatory soil mite Hypoaspis aculeifer the $NOEC_{mortality/reproduction}$ amounted to 1000 mg /kg soil dry weight. These toxicity endpoints are several times higher (see point B.8.1.3 Estimation of concentrations in soil) than predicted environmental concentration in soil of iron pyrophosphate after single application of product BW01 GB (2 mg a.s./kg soil) or six applications (12 mg a.s. /kg soil) of product BW01 GB as recommended in the label. The TERs are then: 500 after single application, and > 83 after multiple application indicating that the risk to non-target arthropods dwelling in soil is low.

B.9.9 Effects on Soil Nitrogen Transformation

Report: Ziółkowska A. 2014, Study of the effect on soil microorganisms - nitrogen transformation according to OECD 216

Study code: 0001/0097/E

Guideline: OECD 216

GLP: yes

Executive Summary:

A study of long-term adverse effects of the test material BW01 GB, the activity of soil microorganisms responsible for nitrogen transformations occurring in aerobic surface soils. The study was based on a comparison of the rate of production of nitrates in the soil exposed to the test material with the rate of production of nitrates in the soil control. Differences in the rate of production of nitrate in the test and control samples at the end of the experiment

(28 days) were 6.12% for a concentration of 5 g/m^2 and 8.16% in the concentration of 30 g/m^2.These differences are ≤ 25% of the test material, therefore, has no long term effect on the conversion of nitrogen in the soil.

A. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: BW01 GB

Description: turquoise pellets

Content of active substance: 3% of iron pyrophosphate

Batch: 032014-P82

Storage: cool and dry place

2. Vehicle and/or positive control: fine quartz sand (particle size 0.1 - 0.5 mm**)**

3. Test system:

Soil: from agricultural areas collected from a depth of 15cm

Source: geographic coordinates 52°27'20"N, 16°56'53"E

Water content of soil: 18,5%

Water holding capacity: 45,4%

pH: 6,0

Total org. C: 3,08% of dry weight of soil

The carbon content of the microbial biomass: 1.41% of the total organic carbon in the soil

Sand content: 65%

4. Environmental conditions

Temperature: 20,5°C - 21,3 °C

pH: 6,0

Water content: 40-60% of maximum capacity

Humidity: 40-48%

B. STUDY DESIGN AND METHODS:

1. Experimental treatments

Using two concentrations of the test material in triplicate (each repeat in a separate container provided test). Concentrations of test material then converted containers per unit area of the test (surface test container is 228 cm^2). The lower concentration was 5 g/m^2 (0.114 g/test container), while the upper 30 g/m^2 (0.684 g/test container). The control was prepared in triplicate. The experiment was conducted in a darkened plastic containers. For each vessel, 400 g of soil, which dealt ¼ test vessel - phase smaller diameter was sufficient to prevent the development of anaerobic conditions. The study lasted 28 days.

2. Observations

During the experiment the following parameters were recorded: soil water content at the beginning of the experiment and throughout the experiment, humidity, temperature. Soil samples from the control and test

concentrations were subjected to chemical analysis for the content of nitrates in the beginning of the experiment and after 7, 14 and 28 days of the study.

3. Statistical calculations

The analysis was based on a comparison of the rate of production of nitrates in the soil exposed to the test material with the rate of production of nitrates in the soil control by F-test at 5% significance level.

4. Deviations from Guideline / Research Plan

In accordance with the study plan, the nitrate content was to be determined in a single repetition of each test concentration. In fact, the assay was done in every repetition of the control and test concentrations. The content of organic carbon in the soil was 3.08% by dry weight of the soil, rather than established 0.5-1.5%. It did not, however, affect the course of the study - the criteria of reliability of the experiment.

II. RESULTS AND DISCUSSION

A. RESULTS

Table 9.9-1 Content of nitrates in the soil

Concentration	The summation of nitrates and nitrites [mg of nitrate and nitrite/kg of dry weight of soil]			The nitrite content [mg nitrite/kg of dry weight of soil]			The nitrate content [mg nitrate/kg of dry weight of soil]		
	Repeat			Repeat			Repeat		
	1	2	3	1	2	3	1	2	3
Analysis of the beginning of the experiment									
Control	19,08	20,24	18,76	0,08	0,08	0,08	19,00	20,16	18,68
5 g/m^2	17,6	18,27	17,08	0,03	0,08	0,03	17,57	18,19	17,04
30 g/m^2	16,87	17,22	16,20	0,03	0,03	0,03	16,83	17,18	16,17
Analysis after 7 days									
Control	17,87	18,22	17,24	0,11	0,11	0,06	17,76	18,11	17,17
5 g/m^2	16,64	16,85	16,01	0,21	0,21	0,16	16,43	16,64	15,85
30 g/m^2	15,87	16,26	15,27	0,16	0,16	0,11	15,71	16,10	15,16
Analysis after 14 days									
Control	14,32	15,13	13,55	0,06	0,06	0,06	14,26	15,07	13,49
5 g/m^2	13,55	14,18	12,81	0,11	0,16	0,11	13,44	14,02	12,70
30 g/m^2	13,13	13,76	12,60	0,21	0,19	0,15	12,92	13,57	12,46
Analysis after 28 days (end of the experiment)									
Control	13,91	14,69	13,04	0,08	0,08	0,03	13,83	14,61	13,00
5 g/m^2	12,97	13,56	12,26	0,08	0,13	0,13	12,88	13,43	12,13
30 g/m^2	12,61	13,14	11,91	0,03	0,03	0,03	12,58	13,11	11,88

Table 9.9-2 The rate of formation of nitrate in the soil at the end of the experiment (28 days)

Concentraction	Production rate of nitrate [mg nitrate / kg dry soil mass / day]				Deviation from the control values
	1	**2**	**3**	**Average**	**Average**
Control	0,49	0,52	0,46	0,49	-
5 g/m^2	0,46	0,48	0,43	0,46	**- 6,12 %**
30 g/m^2	0,45	0,47	0,42	0,45	**- 8,16 %**

III. CONCLUSIONS

Differences in the rate of production of nitrate in the test and control samples at the end of the experiment (after 28 days) were 6.12% for a concentration of 5 g/m^2 and 8.16% in the concentration of 30 g/m^2. These differences are ≤ 25% of the test material, therefore, has no long term effect on the conversion of nitrogen in the soil

RMS comments:
The study of the effect on soil microorganisms - nitrogen transformation was performed in GLP conditions and according to OECD TG 216. 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. No significant differences in production rate of nitrate (> 25%) were observed after application of BW01 GB at a dose of 50 kg/ha and 300 kg/ha.

B.9.10 Risk Assessment for Soil Nitrogen Transformation

Data sufficient for the assessment of risk to soil micro-organisms is provided by a study presented in point B.9.9. Since the maximum number of BW01 GB applications per season is 6, the highest dose tested equals six times the maximum single dose and amounts to 30 g/m^2. In the study, no unacceptable effect of BW01 GB in the highest dose tested on the activity of soil micro-organisms with regard to nitrogen transformation was observed. After 28 days, the differences in the nitrate production pace between the samples tested and the control were ≤ 25%. Thus, the risk to soil micro-organisms connected with nitrogen transformation and resulting from the suggested use of the PPP containing ferric pyrophosphate is acceptable.

RMS comments:
The risk assessment for Soil Nitrogen Transformation was performed in line with recommendations of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final). At the highest dose of a product BW01 GB of 300 kg/ha equal to sum of 6 application of the single dose of 50 kg/ha had no effect on soil micro-organisms producing nitrate in the soil therefore it is concluded that the risk to these organisms due to application of the product BW01 GB as recommended in the label is low and acceptable.

B.9.11 Effects on Terrestrial Non-target Higher Plants

B.9.11.1 Summary of screening data

The PPP containing ferric pyrophosphate does not display a herbicidal effect nor does it impact plant growth regulation, nor does it affect non-target terrestrial 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.9.11.2 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, studies on non-target plants are not required.

B.9.11.3 Extended laboratory studies on non-target plants

Based on the information contained in points B.9.11.1 and B.9.11.2, it has been established that the risk for terrestrial non-target plants is acceptable, thus extended laboratory studies are not necessary.

B.9.11.4 Semi-field and field tests on non-target plants

Based on the information contained in points B.9.11.1 and B.9.11.2, it has been established that the risk for terrestrial non-target plants is acceptable, thus semi-field and field studies are not necessary.

B.9.12 Risk Assessment for Terrestrial Non-target Higher Plants

Both phosphorous and iron are present in the soil in much higher concentrations than in plants and both mostly in insoluble forms. They can be used up/adsorbed by plants only in their water soluble form. Increase in concentration of both Fe and P in the soil (even in bioavailable water soluble form) does not result in their concentrations' increase in plants above the physiological limits. Moreover, the uptake of all plant nutrients including Fe and P is limited by presence and availability of other plant nutrients; N, K, S, Ca, Mg and microelements. So even if there is a surplus of available phosphorous or iron in the soil, their absorption is limited by other nutrients availability [1, Frossard et al. 2000]. If in a very rich soil (very rare situation) all nutrients will be readily available in access this will result in a higher plant growth (mass increase) thus maintaining the concentrations of Fe, P and other nutrients at similar level. Additionally as described below plants regulate uptake of Fe and P reducing it when they are in excess in the soil.

- Plants acquire phosphorus as dissolved phosphate anions from the soil solution. It is probably one of the least available plant nutrients found in the rhizosphere.
- There is a great disproportion in distribution of Pi (inorganic Phosphorous) between plant cells (mM) and soil solution(mM).
- Nearly 80% of applied Pi may be unavailable to plants, in case of Ferric Pyrophosphate extremely low solubility limits this bioavailability even further.
- Solution Pi concentrations in highly weathered soils, sandy soils and alkaline soils are commonly less than 1μM [2, Rae et al. 2003].

Pyrophosphate ions in water solutions are subjected to hydrolysis reaction yielding phosphate ions which are absorbed by plants. This is a spontaneous process due to negative Gibbs free energy of this reaction [3, Colvin et al. 1995]. Phosphate anions are added to the soil on regular basis by farmers as they are a main component of commonly used fertilizers [4, Potarzycki 2009]. Low solubility of ferric pyrophosphate result in even smaller increase of phosphates concentration in soil. In addition, 500 % higher concentration of phosphate in soil result only in two fold increase in phosphorus concentration in plants [5, Fernandes et al. 2012]. This indicates negligible potential of accumulation in plant tissues of phosphates resulting from the use of plant protection product.

Ferric pyrophosphate is a stable molecule that does not dissociate readily (pKA = 22.2), resulting in negligible release of free iron and pyrophosphate ions [6, Gupta et al. 1999]. Iron is absorbed from soil in the form of ferric ions, Fe3+, Fe2+ from solutions and ferrous Fe in the form of chelates . Very low solubility of ferric pyrophosphate causes very small increase of iron content in the soil. Amount of iron in the dry mass of soil does not influence the iron content in plants [7, Fruzińska 2011] due to their physiology. This indicates negligible potential of accumulation in plant tissues of Iron resulting from the use of ppp.

- Iron is an essential microelement for plant growth and development. Soils normally contain high amounts of Fe, but in well aerated and alkaline soils the availability of Fe for plant uptake is very limited.
- Plants have two major problems with iron as a free ion: its insolubility and its phytotoxicity. To ensure iron acquisition from soil and to avoid iron excess in the cells, uptake and homeostasis are tightly controlled. Plants meet the extreme insolubility of oxidized iron at neutral pH values by deficiency inducible chelation and reduction systems at the root surface that facilitate uptake. [1, Frossard et al. 2000]
- Soils rarely contain less than 0.7% Fe(+3) by weight. Thus, a 50 cm layer of soil contains at least 40000 kg Fe per hectare [8, Shenker 2004]
- At the same time soil solution concentration of Fe is very low; 10-10 M Fe, A 50 cm layer of 1 ha of cultivated soil (assuming a 50 cm depth and water content of 40 % by volume), contains as little as 10 mg Fe in its soil solution [8, Shenker 2004]
- A common critical Fe level in plant tissue is 50-70 mg Fe / kg plant dry weight. Thus, assuming the presence of 10 000 kg dry weight plant material per hectare, the crop contains 500- 700 g Fe per hectare [8, Shenker 2004]
- The mass flow of the soil solution to the plant when the annual water consumption of the crop is 10 000 m3 ha-1 will supply it with as little as 50 mg Fe, which is only 0.01% of the plant's minimal need [8, Shenker 2004]
- Yet, plant roots are surrounded by soil containing an amount of iron that exceeds 50 000 times the crop's annual demand for Fe and despite this the level of Iron in plants does not increase to phytotoxic levels. [8, Shenker 2004]

Based on the information presented in points B.9.11.1 and B.9.11.2, it has been established that the risk for non-target terrestrial plants, resulting from the use of the PPP containing ferric pyrophosphate, is acceptable.

Please see also attached files.

1. Frossard E. , Bucher M. , Mächler F. , Mozafar A. and Hurrell R. (2000), Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. J. Sci. Food Agric., 80: 861-879.
2. Anne L. Rae & Donna Glassop et.al "Phosphate transport in plants", *Plant and soil*, 2003, *248*, pp 71-83
3. M.E. Colvin, E. Evleth, Y. Akacem, Quantum chemical studies of pyrophosphate hydrolysis,Journal of the American Chemical Society 117 (1995) 4357-4362.
4. J. Potarzycki, Influence of formulation of phosphorus fertilizer on nitrogen uptake and its efficiency under maize grain cropping, Acta Scientiarum Polonorum seria Agricultura 8 (2009) 3-13.

5. A.M. Fernandes, R. Soratto, Nutrion, dry matter accumulation and partitioning and phosphorus use efficiency of potato grown at different phosphorus levels in nutrient solution, Revista Brasileira de Ciência do Solo 36 (2012) 1528-1537.

6. A. Gupta, N.B. Amin, A. Besarab, S.E. Vogel, G.W. Divie, J.Yee, J.V. Anandan, Dialysate iron therapy: infiusion of soluble ferric pyrophosphate via the dialysate during hemodialysis, Kidney International 55 (1999) 1891-1898.

7. R. Fruzińska, Accumulation of iron in the soli-plant system in a metal industry area, CIVIL AND ENVIRONMENTAL ENGINEERING REPORTS 7 (2011) 59-68.

8. Moshe Shenker and Yona Chen –" Increasing Iron Availability to Crops: Fertilizers, Organo-Fertilizers, and Biological Approaches" October 5, 2004

RMS: The plants require phosphorus and iron as essential elements. Phosphorous is present as a constituent of such compounds as nucleic acids, phospholipids and ATP, and as a metabolite involved in energy transfer, the activation of proteins and the regulation of metabolic processes. The uptake of inorganic phosphate from solution in soil by roots of plants and transport of inorganic phosphate through plant membranes is mediated/controlled by a several transporter proteins coded by specific genes. (Smith at al. 2003), thus it is not govern by the concentration of inorganic phosphates in soil. Iron is also an essential microelement for plant growth and development, e.g. as a component of chlorophyll, therefore in cases of iron deficiency in the soil the iron fertilizer are be applied. Plants have mechanisms to control iron uptake and homeostasis (Morrissey and Guerinot, 2009). The natural concentration of inorganic phosphate and ferric ions in soil are much higher than those caused by application of a product BW01 GB. The plants during evolution have developed mechanisms to control the uptake of phosphate and ferric from the soil, therefore small increases in their concentration in soil due to application of BW01 GB will not affect plants. In addition, due to low solubility in water the bioavailability of phosphate and ferric ions for the plants from ferric pyrophosphate is very low. Therefore justification for not testing toxicity of ferric pyrophosphate to non-target plants is acceptable. Summing up, it is concluded that application of a product BW01 GB in accordance with GAP will not pose an unacceptable risk to non-target plants.

References:

1. Frank W. Smith, Stephen R. Mudge, Anne L. Rae & Donna Glassop: Phosphate transport in plants. Plant and Soil 248: 71–83, 2003.
2. Joe Morrissey and Mary Lou Guerinot: Iron uptake and transport in plants: The good, the bad, and the ionome, Chem Rev. 2009 Oct; 109(10): 4553–4567.

B.9.13 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 terrestrial non-target 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.9.14 Risk Assessment for Other Terrestrial Organisms (Flora and Fauna)

Bases on available data it is assumed that the risk for non-target terrestrial organisms is acceptable.

B.9.15 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.9.1.2.1	[redacted]	2014	Acute Oral Toxicity – Fixed Dose Procedure Study code: 0001/0104/T OECD 420 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.3.1.1/01	[redacted]	2014	Fish Acute Toxicity Test according to OECD Guideline No 203 Study code: 0001/0091/E OECD Guideline 203 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.3.1.2/01	Winkler, J.	2014	Daphnia sp. Acute Immobilization Test according to OECD Guideline No 202 Study code: 0001/0090/E Guideline: OECD 202 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.3.2.1	[redacted]		The study of the long-term and chronic toxicity to fish according to OECD 210 Study code: 0001/0109/E OECD Guideline 210 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.3.2.2	Winkler, J.	2014	Daphnia reproduction test according to OECD 211 Guideline Study code: 0001/0111/E Guideline: OECD 211 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.5.2.1/01	Winkler, J.	2014	Lethal and sublethal effects of molluscicide	Y	BROS Spółka z ograniczoną

			BW01 GB on beetle Poecilus cupreus Study code: 0001/0100/E Guideline: IOBC, BART, EPPO [Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. M.P. Candolfi, S. Blümel, R. Forster et al. (2000)] GLP: yes Unpublished		odpowiedzialnością sp.k.
B.9.5.2.1/02	Pecorari, F.	2015	Evaluation of the chronic effects of the product BW01 GB on the rove beetle Aleochara bilineata Gyllenhal under Laboratory Conditions Study code: BT116/15 Guideline: IOBC/WPRS guidelines (Candolfi M.P. et al., 2000). GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.
B.9.9	Ziółkowska, A.	2014	Study of the effect on soil microorganisms - nitrogen transformation according to OECD 216 Study code: 0001/0097/E Guideline: OECD 216 GLP: yes Unpublished	Y	BROS Spółka z ograniczoną odpowiedzialnością sp.k.