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| REGISTRATION REPORT  Part B  Section 9  Ecotoxicology  Detailed summary of the risk assessment |
| Product code: BAS 743 03 F  Product name(s): **DIVEXO**  Chemical active substance(s):  Ametoctradin 120 g/L  Propamocarb hydrochloride 451 g/L |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT  (authorization of product) |
| Applicant: XXXX  Submission date: October 2023 (update April 2024)  Evaluation date: May 2024  MS Finalisation date: November 2024 |

Version history

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| --- | --- |
| When | What |
| October 2023 | Initial dRR – XXXX DocID 2023/2035810 |
| April 2024 | dRR update – XXXX Doc ID 2024/2010830   * 9.1 Critical GAP and overall conclusions update * 9.2 Effects on birds update * 9.3 Effects on terrestrial vertebrates other than birds update * 9.6 Effects on bees update |
| May 2024 | zRMS finalised dRR evaluation |
| November 2024 | Revised version addressing the comments resived |

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# Ecotoxicology (KCP 10)

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| **Review Comments:**  This document describes the acceptable use conditions required for registration of DIVEXO (BAS 743 03 F), a SC formulation containing 120 g/L Ametoctradin and 451 g/L Propamocarb hydrochloride (=378 g/L Propamocarb) for the use as fungicide in potato, onion, tomato and aubergine.  It should be noted that for the GAPs minor uses according to Article 51 no PEC calculations following zonal requirements are provided in this dossier B8. Thus, for those uses, the risk to aquatic and soil organisms is not covered in this report.  This Part B document only reviews data and additional information that has not previously been considered within the EU review process.  Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey. |

## Critical GAP and overall conclusions

Table ‑: Table of critical GAPs

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Use-No. \* | Member state(s) | Crop and/or situation  (crop destination / purpose of crop) | F, Fn, Fpn G, Gn, Gpn or  I \*\* | Pests or Group of pests controlled  (additionally: developmental stages of the pest or pest group) | Application | | | | Application rate | | | PHI  (days) | Remarks:  e.g. g saf­ener/ syner­gist per ha | Conclusion | | | | | | |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product/ha  a) max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha  a) max. rate per appl.  b) max. total rate per crop/season | Water L/ha  min/max | Birds | Mammals | Aquatic organisms | Bees | Non-target arthropods | Soil organisms | Non-target plants |
| Zonal uses (field or outdoor uses, certain types of protected crops) | | | | | | | | | | | | | | | | | | | | |
| 1 | BE, IE, NL | Potato (including seed potatoes) (SOLTU) | F | *Phytophthora infestans* (PHYTIN) | SP | BBCH 21-89 | a) 3  b) 3 | 5 | a) 2  b) 6 | a) 0.24(\*) + 0.902(\*\*)  b) 0.72(\*) + 2.706(\*\*) | 100/1000 | 7 | Spray interval: 5-10 days  Water volume:  NL: 150/400 L/ha  IE: 200/400 L/ha  Applications only every 2nd year | A | A | A | A | A | A | A |
| 2 | PL HU, RO, SI, SK AT, CZ, DE | Potato (including seed potatoes) (SOLTU) | F | *Phytophthora infestans* (PHYTIN) | SP | BBCH 21-89 | a) 2  b) 2 | 5 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 200/400 | 7 | Spray interval: 5-10 days  Dose rate range for HU, RO, SI, SK: 1,5-2 L/ha | A | A | A | A | A | A | A |
| 3 | BE, IE, NL, PL, RO | Onion  (ALLCE), Garlic (ALLSA) | F | *Peronospora destructor*  (PERODE) | SP | BBCH 14 – 39 ~~49~~ | a) 2  b) 2 | 5 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 200/1000 | 7 | Spray interval: 5-10 days  Water volume:  NL, PL: 200/800 L/ha  IE. 200/700 L/ha Applications only every 2nd year | A | A | A | A | A | A | A |
| 4 | AT, CZ, DE, HU, SK, SI | Onion  (ALLCE), Garlic (ALLSA) | F | *Peronospora destructor*  (PERODE) | SP | BBCH 14 - 49 | a) 1  b) 1 | NA | a) 2  b) 2 | a) 0.24(\*) + 0.902(\*\*)  b) 0.24(\*) + 0.902(\*\*) | 200/1000 | 7 |  | A | A | A | A | A | A | A |
| 5 | PL,  HU, RO, SK, SI | Tomato / Aubergine  (LYPES) / (SOLME) | F | Phytophthora infestans (PHYTIN) | SP | BBCH ~~21~~  40-89 | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 150/500 | 1 | Spray interval: 7-10 days | A | A | A | A | A | A | A |
| Minor uses according to Article 51 (field uses) \*\*\* | | | | | | | | | | | | | | | | | | | | |
| 6 | NL | Floriculture crops DTG .2)  (unprotected culture) | F | *Peronospora sp* (PEROSP)  *Phytophthora spp (*PHYTSP) | Foliar treatment | BBCH 12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA |  |  |  |  |  |  |  |  |
| 7 | NL | Avenue trees | F | *Peronospora sp* (PEROSP) | Foliar Treatment | BBCH 12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA |  |  |  |  |  |  |  |  |
| 8 | NL | Climbing Plants | F | *Peronospora sp* (PEROSP) | Foliar Treatment | BBCH 12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA |  |  |  |  |  |  |  |  |
| 9 | NL | Conifers (incl. Christmas trees) | F | *Peronospora sp* (PEROSP) | Foliar Treatment | BBCH-12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA |  |  |  |  |  |  |  |  |
| 10 | NL | Ornamental shrubs (incl. roses) | F | *Peronospora sp* (PEROSP) | Foliar Treatment | BBCH-12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA |  |  |  |  |  |  |  |  |
| 11 | NL | Heather | F | *Phytophthora spp (*PHYTSP) | Foliar Treatment | BBCH-12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA |  |  |  |  |  |  |  |  |
| 12 | NL | Forest trees and hedging plants | F | *Peronospora sp* (PEROSP) | Foliar Treatment | BBCH-12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA |  |  |  |  |  |  |  |  |
| 13 | NL | Fruit trees and shrubs (nursery) | F | *Peronospora sp* (PEROSP) | Foliar Treatment | BBCH-12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA | Ornamental crop not for fruit production |  |  |  |  |  |  |  |
| 14 | NL | Perennial crops (nursery) | F | *Peronospora sp* (PEROSP) | Foliar Treatment | BBCH-12-59 (Apr-Sep) | a) 2  b) 2 | 7 | a) 2  b) 4 | a) 0.24(\*) + 0.902(\*\*)  b) 0.48(\*) + 1.804(\*\*) | 500 | NA | Tree nursery green non-woody perennials crops not for fruit production (e.g. Papaver, Geranium, Erigeron, Coydalis, Viola, Veronica, …) |  |  |  |  |  |  |  |

\* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

\*\* F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

\* *For further details on concerned ornamental crops intended in The Netherlands and better understanding of the hierarchical classification followed in this country, please refer to the Definition List of Application Areas of Crop Protection Products (DTG list) from Ctgb which contains the standard terms for application areas of crop protection products for the Legal Instructions for Use (WG). The DTG list contains agricultural and horticultural crops, public green areas, uncultivated areas and terms for non-professional use*. *Please, refer to the following direct link:*

[Definitielijst Toepassingsgebieden Gewasbeschermingsmiddelen 2.2 | Toetsingskader gewasbeschermingsmiddel | College voor de toelating van gewasbeschermingsmiddelen en biociden (ctgb.nl)](https://eur02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.ctgb.nl%2Fdocumenten%2Ftoetsingskader-gewasbeschermingsmiddelen%2F2019%2F06%2F01%2Fdefinitielijst-toepassingsgebieden-gewasbeschermingsmiddelen-dtg-2.2&data=05%7C02%7Cgemma.peidro-saperas%40basf.com%7C8e21ff0d11b14e0f993808dc5560fa95%7Cecaa386bc8df4ce0ad01740cbdb5ba55%7C0%7C0%7C638479122208585391%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C0%7C%7C%7C&sdata=X5nJSR5EjNYklrxPd40eI0rz3EiCufCFeOyYiD9FN1g%3D&reserved=0)

*For the presentation of risk assessments only requested in the concerned Member State (The Netherlands), reference is made to the National Addendum (i.e. chronic risk assessments for bees based on EFSA guidance (2013, updated 2014)*

Explanation for column 15 – 21 “Conclusion”

|  |  |
| --- | --- |
| A | Acceptable, Safe use |
| R | Further refinement and/or risk mitigation measures required |
| C | To be confirmed by cMS |
| N | No safe use |

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| **Remarks**  **table:** | (1) Numeration necessary to allow references  (2) Use official codes/nomenclatures of EU  (3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)  (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application  (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named  (6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench  Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated |  | (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3‑8263-3152-4), including where relevant, information on season at time of application  (8) The maximum number of application possible under practical conditions of use must be provided  (9) Minimum interval (in days) between applications of the same product.  (10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products  (11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).  (12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.  (13) PHI - minimum pre-harvest interval  (14) Remarks may include: Extent of use/economic importance/restrictions |

### Overall conclusions

#### Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

##### Effects on birds (KCP 10.1.1)

*Dietary risk assessment*

In the screening step risk assessment, all TERA values and all TERlt values for Ametoctradin exceed the trigger set by Commission Regulation (EU) 546/2011 for acceptability of effects. For the active substance Propamocarb-HCl and the formulation BAS 743 03 F acceptable dietary acute and long-term risk for birds is indicated at Tier 1 level except for the use in fruiting vegetables where an acceptable long-term risk was demonstrated at higher tier level including considerations of combined exposure.

*Drinking water risk assessment*

Following EFSA/2009/1438, the puddle scenario is considered relevant for the proposed use pattern. Since the ratios of the effective application rates to the relevant toxicity endpoints are below the relevant trigger values for both Ametoctradin and Propamocarb-HCl, a quantitative risk assessment for the proposed use pattern of BAS 743 03 F is not necessary for the puddle scenario. In conclusion the proposed use pattern of BAS 743 03 F does not pose a risk to birds via up-take of contaminated drinking water.

*Secondary poisoning and biomagnification*

The log Pow of Propamocarb-HCl does not exceed the trigger value of 3. However, the log Pow of the active substance Ametoctradin was determined to be 4.4, which triggers an assessment of the potential risk from secondary poisoning. According to the tier 1 risk assessment for earthworm- and fish-eating birds, the TER values for Ametoctradin are above the trigger value of 5, indicating an acceptable risk. The potential for bioaccumulation of Ametoctradin and Propamocarb-HCl was considered as low in the respective EU reviews and therefore further evaluation on biomagnification is not necessary.

***Overall conclusion***

**It can be concluded that the risk to birds from the application of BAS 743 03 F according to good agricultural practice is acceptable.**

##### Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

*Dietary risk assessment*

In the screening step risk assessment, all TERA values and all TERlt values for Ametoctradin exceed the trigger set by Commission Regulation (EU) 546/2011 for acceptability of effects. ~~For the active substance Propamocarb-HCl, an acceptable dietary acute risk for mammals is indicated at Tier 1 level while an acceptable dietary long-term risk for Propamocarb-HCl is indicated at higher-tier level. For the formulation BAS 743 03 F an acceptable dietary acute risk for mammals is indicated at higher tier level including considerations of combined exposure.~~

All acute TER values exceed the relevant triggers in the Tier 1 risk assessment for propamocarb-HCl except for the minor use (surrogate crop: orchards BBCH 10-19).

Based on the higher tier chronic risk assessment for propamocarb-HCl, where the deposition factor and ~~DT~~~~50~~ ~~in plants~~ PD for voles were modified, the TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects except for uses in fruiting vegetables at BBCH 11-39, multiple applications in onions BBCH ≥ 40, ornamentals BBCH 40-49, and for surrogate crops scenarios: orchards BBCH 10-40 and bush and cane fruit BBCH 10-39.

*Drinking water risk assessment*

Following EFSA/2009/1438, the puddle scenario is the one relevant for mammals. Since the ratios of the effective application rates to the relevant toxicity endpoints are below the relevant values for both Ametoctradin and Propamocarb-HCl, a quantitative risk assessment for the proposed use pattern of BAS 743 03 F is not necessary for the puddle scenario. In conclusion the proposed use pattern of BAS 743 03 F does not pose a risk to mammals via uptake of contaminated drinking water.

*Secondary poisoning and biomagnification*

The log Pow of Propamocarb-HCl does not exceed the trigger value of 3. However, the log Pow of the active substance Ametoctradin was determined to be 4.4, which triggers an assessment of the potential risk from secondary poisoning. According to the tier 1 risk assessment for earthworm- and fish-eating mammals, the TER values for Ametoctradin are above the trigger value of 5, indicating an acceptable risk. The potential for bioaccumulation of Ametoctradin and Propamocarb-HCl was considered as low in the respective EU reviews and therefore further evaluation on biomagnification is not necessary.

***Overall conclusion***

**It can be concluded that the risk to mammals from the application of BAS 743 03 F according to good agricultural practice is acceptable.**

##### Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

In the EU there are currently no guidance documents by EFSA on how to conduct risk assessments for amphibians and reptiles. Nevertheless, the latest EFSA guidance document on aquatic organisms (EFSA 2013) provides the recommendation to look for the availability of studies on the toxicity of the active substances to amphibians and, if available, to take such toxicity data into consideration.

For both active substances contained in the formulation BAS 743 03 F, i.e. Ametoctradin and Propamocarb-HCl, there are no studies available, neither in the literature nor unpublished reports by the notifier, on their toxicity to amphibians or reptiles. Therefore, due to the lack of a standard risk assessment and of data on the toxicity of the active substances to amphibian and reptiles, a regulatory risk assessment for these organisms is not applicable at this time.

#### Effects on aquatic organisms (KCP 10.2)

For Ametoctradin, the acute studies conducted with the active substance resulted in endpoints which are greater than the water solubility of the compound under test conditions. However, higher concentrations of the active substance could be achieved by testing the formulated product BAS 650 00 F (nominal content of Ametoctradin: 200 g a.s./L). Therefore, it was decided to include studies with the formulated product BAS 650 00 F, indicating an overall low to moderate toxicity. In the risk assessment, PEC/RAC ratio calculations are based on the endpoints derived with the formulated product (based on the content of the active substance). For Propamocarb, the EU agreed endpoints for acute and long-term toxicity to aquatic organisms are used in the risk assessment. For the formulated product BAS 743 02 F, studies on the acute toxicity to fish and *Daphnia magna* as well the effects on green algae are available; in addition to measured toxicity data, mixture toxicity is also calculated based on the Concentration Addition (CA) model.

For the active substance Ametoctradin, the calculated PEC/RAC ratios are below the trigger of 1 at FOCUS Step 3 for all intended uses indicating an acceptable risk for all groups of aquatic organisms without the necessity of mitigation measures.

For Ametoctradin relevant metabolites, the calculated PEC/RAC ratios are significantly below the trigger of 1 at FOCUS Step 1; they are thus considered not to be of ecotoxicological relevance.

For the active substance Propamocarb-HCl, the calculated PEC/RAC ratios are below the trigger of 1 at FOCUS Step 2 for all intended uses of BAS 743 03 F.

The active substance Ametoctradin contributes by more than 90% to the chronic toxicity of the formulation BAS 743 03 F to fish and aquatic invertebrates; thus the risk assessment for these trophic level is based on the single-substance toxicity data of Ametoctradin. No “driver” of acute mixture toxicity is identified for fish, aquatic invertebrates and algae; thus, any potential risk due to the acute toxicity of BAS 743 03 F is addressed in a mixture risk assessment following the Risk Quotient Approach (RQ). For BAS 743 03 F the calculated RQmix ratios are below the trigger of 1 at FOCUS Step 1 for all intended uses of the formulated product. In addition, the calculated PEC/RAC ratios based on measured formulation endpoints for drift entry are significantly below the trigger of 1 indicating an acceptable risk for all intended uses of BAS 743 03 F.

It should be noted that for the GAPs minor uses according to Article 51 no PEC calculations following zonal requirements are provided in this dossier B8. Thus, for those uses, the risk to aquatic organisms is not covered in this report.

#### Effects on bees (KCP 10.3.1)

The risk assessment has been performed according to SANCO/10329/2002 rev 2 final, since the new EFSA GD *“Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)”* (EFSA Journal 2013; 1187):3295) has not been adopted by the Standing Committee on Plants, Animals, Food and Feed.

The acute risk to honeybees from the intended uses of BAS 743 03 F is assessed using the maximum single application rate and the relevant LD50 values for the active substances Propamocarb-HCl and Ametoctradin and the formulated product BAS 743 03 F to calculate hazard quotients (HQ) for oral exposure (QHO) and contact exposure (QHC). The hazard quotients for both active substances and the formulated product for acute oral and acute contact exposure of honeybees are below the Commission Regulation (EU) 546/2011 trigger value of 50, indicating an acceptable risk.

In addition, data on chronic oral toxicity to adult honeybees and on oral toxicity to honeybee larvae for the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) are available. These studies indicate low toxicity to bees and meet the current data requirements (Commission Regulation (EU) No. 283/2013 and 284/2013). However, under the current risk assessment scheme (SANCO/10329/2002) there is no requirement to conduct a risk assessment with these endpoints.

Taking all data together, it can be concluded that the proposed uses of BAS 743 03 F pose no unacceptable risk to honeybees, if applied according to the recommended use patterns.

Some CEU MSs require evaluation according to EFSA 2013. This approach is still not harmonised, but it was discussed at the last meeting of the central zone in the field of ecotoxicology (Warsaw, 12.2023), where it was agreed to present an assessment in the Core in accordance with EFSA 2013. Thus, required chronic risk assessment was provided. ~~Currently, the minutes of the meeting are still in the course of zonal consultations.~~ For Poland, a chronic risk assessment is not required. ~~Nevertheless, the Applicant, as a result of commenting process, may be asked to supplement the dossier with a risk assessment for bees in accordance with EFSA 2013.~~

#### Effects on arthropods other than bees (KCP 10.3.2)

The testing and risk assessment strategy used here follow the approach recommended in the ESCORT 2 guidance document, ESCORT 3, and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002)*.* The risk assessment for BAS 743 03 F is based on tests with the standard test species *Typhlodromus pyri* and *Aphidius rhopalosiphi*.

An acceptable in- and off-field risk for *Typhlodromus pyri* and *Aphidius rhopalosiphi* was found based on glass plate laboratory data. Therefore, an overall acceptable risk to non-target arthropods for all intended uses of BAS 743 03 F to field crops (potato, onion, tomato and aubergine) can be concluded.

#### Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

The evaluation of the risk for earthworms, other non-target soil organisms (meso- and macrofauna) and soil microorganisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

***Effects on non-target soil meso- and macrofauna***

All TER values for BAS 743 03 F, ~~the active substance Ametoctradin, and relevant metabolites as well as the active substance Propamocarb-HCl~~ for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5.

**Therefore, it can be concluded that BAS 743 03 F poses no unacceptable risk to earthworms or other soil meso- and macrofauna when applied according to the proposed uses.**

***Effects on soil microbial activity***

Studies on effects to soil microorganisms have been carried out with the active substance Ametoctradin, the Ametoctradin relevant soil metabolites, the active substance Propamocarb-HCl and with the formulation BAS 743 03 F.

The potential risk to soil micro-organisms was assessed by comparing the maximum PECsoil values with the maximum concentration with effects ≤ 25%. For the formulation BAS 743 03 F, the two active substances Ametoctradin and Propamocarb-HCl, as well as for the relevant Ametoctradin metabolites, the maximum concentrations with effects < 25% (SANCO/10329/2002 trigger) are all by far above the PECsoil values derived from the maximum recommended application rate.

**Therefore, it can be concluded that the use of BAS 743 03 F will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.**

It should be noted that for the GAPs minor uses according to Article 51 no PEC calculations following zonal requirements are provided in this dossier B8. Thus, for those uses, the risk to soil organisms is not covered in this report.

#### Effects on non-target terrestrial plants (KCP 10.6)

The risk assessment was based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). BAS 743 03 F is a fungicide and is therefore not expected to have significant herbicidal activity. Hence, a Tier 1 assessment was conducted, using the available screening data.

A study on the effects of BAS 743 03 F exposure on seedling emergence and vegetative vigour of terrestrial higher plants was conducted. The results showed that applications up to a rate of 3.85 L BAS 743 03 F/ha caused no reduced seedling emergence and plant fresh weight, and no symptoms of phytotoxicity were observed for any of the seven terrestrial plant species tested.

The Tier 1 risk assessment based on screening data demonstrates an acceptable risk to non-target terrestrial plants for all intended uses of BAS 743 03 F.

**Based on the risk assessment it can be concluded that the proposed uses of BAS 743 03 F pose no unacceptable risk to non-target plants, if applied according to the recommended use patterns. Particular precautions to reduce the environmental concentrations resulting from BAS 743 03 F applications are not required for the protection of terrestrial non-target plants.**

#### Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant.

### Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

Table 9.1‑2: Critical use pattern of BAS 743 03 F grouped according to worst-case application

| **Grouping according to worst-case application** | | | | |
| --- | --- | --- | --- | --- |
| **Area** | **Group** | **Intended uses** | **Relevant use parameters for grouping** | **Relevant parameter or value for sorting** |
| Birds | Potatoes, onions (Bulbs and onion like crops), tomato/aubergine (crop group fruiting vegetables), ornamentals, orchards, bush and cane fruit | 1 – 3 x 2 L product/ha | Crop group according to EFSA/2009/1438 | The worst-case application scenario is selected within each crop group separately. |
| Birds secondary poisoning | 1 – 3 x 2 L product/ha | Worst-case PEC values resulting from calculations for single or multiple application following FOCUS surface water modelling | Worst-case PECSW |
| Mammals | Potatoes, onions (Bulbs and onion like crops), tomato/aubergine (crop group fruiting vegetables), ornamentals, orchards, bush and cane fruit | 1 – 3 x 2 L product/ha | Crop group according to EFSA/2009/1438 | The worst-case application scenario is selected within each crop group separately. |
| Mammals secondary poisoning | 1 – 3 x 2 L product/ha | Worst-case PEC values resulting from calculations for single or multiple application following FOCUS surface water modelling | Worst-case PECSW |
| Aquatic organisms | Potatoes, onions (Bulbs and onion like crops), tomato/aubergine (crop group fruiting vegetables), ornamentals | 1 – 3 x 2 L product/ha | Worst-case PEC values resulting from calculations for single or multiple application following FOCUS surface water modelling | Worst-case PECSW |
| Bees | Maximum single application rate | 1 x 2 L product/ha | Maximum single application rate | 1 – x 2 L product/ha |
| Non-target arthropods | Field crops | 1 – 3 x 2 L product/ha | Worst-case application pattern (i.e. maximum application rate, maximum number of applications), crop group according to ESCORT 2 | Highest multiple application rate in potatoes: 3 x 2.0 L product/ha |
| Soil macro- and mesofauna, soil microorganisms | Onion/cucurbits | 1 – 3 x 2 L product/ha | Worst-case PEC values | Worst-case PEC values calculated for onions/cucurbits |
| Non-target plants | Field crops | 1 x 2 L product/ha | Maximum single application rate | 1 x 2 L product/ha |

### Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of BAS 743 03 F is indicated in the tables for both active substances.

Table 9.1‑3 Metabolites of Ametoctradin

| Metabolite | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
| --- | --- | --- | --- | --- |
| M650F01 |  | 249.3 | Soil: max. 53.9 %  Water: max. 24.4 %  Sediment: max. 1.5 % | **Aquatic**  Metabolite relevant for RA: yes  **Terrestrial**  Metabolite relevant for RA: no 2) RA conducted: no 2) |
| M650F02 |  | 235.3 | Soil: max. 13.0 %  Water: max. 10.2 %  Sediment: max. 3.0 % | **Aquatic**  Metabolite relevant for RA: no 1) RA conducted: no 1)  **Terrestrial**  Metabolite relevant for RA: no 2) RA conducted: no 2) |
| M650F03 |  | 221.2 | Soil: max. 57.0 %  Water: max. 55.3 %  Sediment: max. 20.8 % | **Aquatic**  Metabolite relevant for RA: yes RA conducted: yes  **Terrestrial**  Metabolite relevant for RA: yes RA conducted: yes |
| M650F04 |  | 207.2 | Soil: max. 55.7 %  Water: max. 20.0 %  Sediment: max. 6.1 % | **Aquatic**  Metabolite relevant for RA: yes RA conducted: yes  **Terrestrial**  Metabolite relevant for RA: yes RA conducted: yes |

Abbreviations: RA = risk assessment

1) M650F02 is an intermediate metabolite with similar chemical structure as M650F01 and/or M650F03 and hence it is covered by aquatic risk assessment of these metabolites (for details please refer the updated DAR of ametoctradin (2010))

2) Following the EFSA Journal 2012;10(11):2921, no soil assessment is required for the Ametoctradin metabolites M650F01 and M650F02

## Effects on birds (KCP 10.1.1)

### Toxicity data

Avian toxicity studies have been carried out with Ametoctradin and Propamocarb. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of BAS 743 03 F were not evaluated as part of the EU assessment of Ametoctradin or Propamocarb. A new acute oral study on the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) with Bobwhite quail is submitted with this application and is listed in Appendix 1 and summarised in Appendix 2. Since differences in co-formulants and/or their concentration between both formulations are considered minimal and both formulations are SC (suspension concentrates), it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission. In addition, several ecotoxicological bridging studies have been performed with the formulation BAS 743 03 F on aquatic invertebrates (*Daphnia magna*, BAS Doc ID 2022/2033730), adult honey bees (*Apis mellifera*, acute oral and contact, XXXX Doc ID 2022/2033729), non-target terrestrial arthropods (*Aphidius rhopalosiphi,* XXXX Doc ID 2022/2033732) and chronic earthworms (*Eisenia fetida*, XXXX Doc ID 2022/2033731) indicating no increased toxicity of BAS 743 03 F compared to BAS 743 02 F based on the content of active substance.

The provision of further chronic data on the formulation BAS 743 03 F are not necessary as active substance data on the toxicity to birds is used and additional formulation data are not considered essential. Direct exposure of birds to applications of the formulation is considered unlikely, because at the time of application and for a short period thereafter, most birds will leave the immediate vicinity of spray operations in response to the human disturbance. Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since dietary exposure is the main route of exposure, toxicity data for the active substances are used in preference to data from tests with the formulated material. Furthermore, data on mammals do not indicate higher toxicity of the formulation compared to the active substance and no effects were observed for the active substances.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process, plus the endpoint for the formulation has been taken into account.

**Active substances**

An overview of the EU agreed endpoints for Ametoctradin is given in . In case the selection of studies and endpoints for the risk assessment deviates from the results of the EU review process, justifications are provided below.

Table ‑: Ametoctradin (BAS 650 F): Endpoints relevant for the risk assessment for birds

| Species | Substance | Exposure  System | Results | Reference  [XXXX DocID] |
| --- | --- | --- | --- | --- |
| *Colinus virginianus* | Ametoctradin | Oral 1 d Acute | LD50 > 2000 mg/kg b.w. | EFSA Journal 2012, 10(11): 2921; 2006/1038389 |
| *Anas platyrhynchos* | Ametoctradin | Oral 1 d Acute | LD50 > 2000 mg/kg b.w. | EFSA Journal 2012, 10(11): 2921; 2006/1038390 |
| *Colinus virginianus* | Ametoctradin | Dietary Reproductive toxicity | NOEL = 115.2 mg/kg b.w./d | EFSA Journal 2012, 10(11): 2921;  2008/1023023,  2008/1071959 1),  2009/1079840 1) |
| *Anas platyrhynchos* | Ametoctradin | Dietary Reproductive toxicity | NOEL = 187.8 mg/kg b.w./d | EFSA Journal 2012, 10(11): 2921;  2007/1050786  2009/1079841 2**),**  2009/1111606 2**)** |
| **Endpoint used for acute risk assessments** | **Ametoctradin** | **Oral, 1 d**  **Acute** | **LD50 (extrapolated, geometric mean) = 3776 mg/kg b.w. 3)** | **Extrapolation and geometric mean of quail and mallard LD50 values**  **2006/1038389, 2006/1038390** |
| **Endpoint used for reproductive risk assessments** | **Ametoctradin** | **Dietary Reproductive toxicity** | **NOEL = 115.2 mg a.s./kg b.w./d** | **EFSA Journal 2012, 10(11): 2921;**  **2008/1023023,**  **2008/1071959 1),**  **2009/1079840 1)** |

1) Amendments to the report (XXXX, 2008/1023023). Note that this report was only slightly modified (a mistake in the table order and typing errors were removed).

2) Amendments to the report (XXXX, 2007/1050786). Note that this report was only slightly modified (a mistake in the table order and typing errors were removed).

3) New endpoint. For details see section 9.2.1.1.

Table 9.2‑2 Propamocarb-HCl: Endpoints relevant for the risk assessment for birds

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Colinus virginianus* | Propamocarb-HCl | Oral 1 d Acute | LD50 > 1842 mg/kg b.w.  LD50 (extrapolated) = 3477.7 mg/kg b.w. 1) | EFSA Scientific Report 2006, 78, 1-80 |
| *Colinus virginianus* | Propamocarb-HCl | Dietary Reproductive toxicity | NOEL = 105 mg/kg b.w./d | EFSA Scientific Report 2006, 78, 1-80 |

1) New endpoint. For details see section 9.2.1.1.

**Metabolites**

In the assessment of the metabolites of Ametoctradin carried out in the EU evaluation of this active substance (EFSA (2012) Scientific report 10 (11) 2921: Conclusion on the peer review Ametoctradin (BAS 650 F) it was concluded that it was not necessary to carry out a specific risk assessment for birds and mammals for any of the Ametoctradin metabolites. Therefore, no risk assessment for metabolites is presented in this dossier.

**Mixture toxicity**

An acute oral toxicity test with the similar formulation BAS 74302 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) is available indicating that the product is non-toxic by oral route in birds, *i.e.* LD50 > 2000 mg product/kg bw.

Table 9.2‑3 Formulation BAS 743 03 F: Endpoints relevant for the risk assessment for mammals

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Colinus virginianus* | BAS 743 02 F\* | Oral 1 d Acute | LD50 > 2000 mg/kg bw  **LD50 (extrapolated, geometric mean) = 3776 mg/kg b.w.1)** | New study  2022/2033724 |

1) Since no mortality occurred in the acute oral gavage study and the number of birds tested were 10, the relevant extrapolation factor of 1.888 (EFSA/2009/1438, Table 1, section 2.1.2) was applied to the endpoint of LD50 > 2000 mg/kg b.w..

\* Study was conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl

Nevertheless, in line with EFSA/2009/1438, for the assessment of acute effects (mortality) from the simultaneous exposure of birds to residues of Propamocarb and Ametoctradin (considered to be the only potential toxicants contained in the formulation), a surrogate LD50 (mix) is additionally derived. The surrogate LD50 (mix) is calculated assuming dose additivity of toxicity by using the following equation:

With:

X (a.s.i) = fraction of active substance [i] in the mixture

LD50 (a.s.i) = acute toxicity value for active substance [i] (pragmatically, NOEL (a.s.i) may be inserted, too)

In addition to acute, the long-term risk from combined exposure of birds and mammals to active substances needs to be addressed via the Concentration Addition Model.

The estimation of the LD50 (mix) and NOEL (mix) is shown in the following tables**.**

Table 9.2‑4 Estimation of LD50 for the mixture assuming dose additivity

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Active substance** | **Concentration of each active substance in ~~formulation~~ BAS 743 02 F**  **[g a.s.i/L]** | **X (a.s.i) in the mixture** | **LD50 [mg a.s.i/kg bw]** | **Σ [X (a.s.i)/LD50 (a.s.i)]** | **LD50 (mix) [Σ mg a.s.i/kg bw]** |
| Ametoctradin | 137 | 0.21 | 3776 | < 2.8E-04 | 3536.4 |
| Propamocarb-HCl | 515 1) | 0.79 | 3477.7 1) |

1) Expressed as Propamocarb-Hydrochloride

Table 9.2‑5 Estimation of NOEL for the mixture assuming dose additivity

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Active substance** | **Concentration of each active substance in ~~formulation~~ BAS 743 02 F**  **[g a.s.i/L]** | **X (a.s.i) in the mixture** | **NOEL [mg a.s.i/kg bw]** | **Σ [X (a.s.i)/NOEL (a.s.i)]** | **NOEL (mix) [Σ mg a.s.i/kg bw]** |
| Ametoctradin | 137 | 0.21 | 115.2 | 9.35E-03 | 107 |
| Propamocarb-HCl | 515 1) | 0.79 | 105 1) |

1) Expressed as Propamocarb-Hydrochloride

Measured LD50 values, if available, should only be replaced in the risk assessment by modelled data if a significant change of the predicted risk is to be expected. To achieve a basis for a comparison of single active substance and mixture toxicity in terms of potential risk, a “tox per fraction” quotient can be calculated for each active substance and compared to the corresponding quotient for the mixture:





Table 9.2‑6 Comparison of “tox per fraction (a.s.i)” and “tox per fraction (mix)” for acute toxicity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Active substance** | **LD50 (a.s.i) [mg a.s./kg bw]** | **X (a.s.i) in the mixture** | **Tox per fraction:**  **[LD50 (a.s.i)/X (a.s.i)]** | **Contribution to overall toxicity  [%] 1)** |
| LD50 (mix) | 3776 | 1.0 | 3776 | - |
| Ametoctradin | 3776 | 0.21 | 17981 | 19.7 |
| Propamocarb-HCl | 3477.7 | 0.79 | 4402.2 | 80.3 |

1) Deviation [%] = 100-[(tox per fraction (a.s.*i*) - tox per fraction (mix)]/tox per fraction (a.s.*i*) × 100

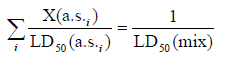
Table 9.2‑7 Comparison of “tox per fraction (a.s.*i*)” and “tox per fraction (mix)” for long-term/reproductive toxicity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Active substance** | **NOEL(a.s.i) [mg a.s./kg bw]** | **X (a.s.i) in the mixture** | **Tox per fraction:**  **[NOEL (a.s.i)/X (a.s.i)]** | **Contribution to overall toxicity  [%] 1)** |
| NOEL (mix) | 107 | 1.0 | 107 | - |
| Ametoctradin | 115.2 | 0.21 | 548.6 | 19.5 |
| Propamocarb-HCl | 105 | 0.79 | 132.9 | 80.5 |

1) Contribution to overall toxicity [%] = 100-[(tox per fraction (a.s.*i*) - tox per fraction (mix)]/tox per fraction (a.s.*i*) × 100

None of the active substances contribute to ≥ 90 % to the acute or long-term/reproductive toxicity of formulationBAS 743 03 F. Therefore, acute and long-term risk assessments for mammals are presented for both active substances and the formulated product.

An acute endpoint from a study with the similar formulation BAS 743 02 F is available. Following Appendix B of the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA/2009/1438), the LD50 value is compared with the predicted mixture toxicity assuming dose additivity, according to the following formulation:



With:

X(a.s.i) = fraction of active substance [i] in the mixture (here: formulation)

LD50(a.s.i) = acute toxicity value for active substance [i]

LD50(mix) = measured acute toxicity value for the mixture (here: formulation)

The resulting comparison between the measured LD50 based on the LD50 values for birds for Ametoctradin (LD50 =3776 mg a.s./kg bw) and Propamocarb (LD50 = 3477.7 mg a.s./kg bw) with the predicted mixture toxicity assuming dose additivity is as followed:

The left-hand side of the equation (predicted mixture toxicity) is:

< 0.00028

And the right hand side of the equation (measured toxicity for comparison) is:

1 / 2281.3 (product endpoint corrected for a.s. content and product density of BAS 743 02 F) = <0.0004

A greater value on the right side of the equation, as is the case here, indicates that the measured toxicity of a formulation is higher than predicted. In this case the use of the measured LD50 for the formulation is recommended for the first-tier assessment**.**

#### Justification for new endpoints

Acute - The EU agreed endpoints for Ametoctradin (EFSA Journal 2012, 10: 2921) and Propamocarb (EFSA Scientific Report 2006, 78, 1-80) were derived using the former guidance on birds and mammals (SANCO/4145/2000). Following the recommendations of current guidance (EFSA/2009/1438) a new endpoint for the acute risk assessment is justified.

Because no mortality occurred in the acute oral gavage studies in quail for both active substances and the number of birds tested were 10, the relevant extrapolation factor is 1.888 (EFSA/2009/1438, Table 1, section 2.1.2). Using this extrapolation factor to the endpoint of each of the two acute studies (LD50 > 2000 mg/kg b.w.) the extrapolated endpoint is LD50 (extrapolated) = 3776 mg/kg b.w for Ametoctradin and LD50 (extrapolated) = 3477.7 mg/kg b.w. for Propamocarb.

### Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

**Proposed use pattern for the risk assessments**

The proposed use pattern for the use of BAS 743 03 F is summarized in the table at the beginning of the ecotoxicology chapter (section 9.1).

To achieve a concise risk assessment, the risk envelope approach is applied. As for Ametoctradin risk acceptability is indicated at screening step, the risk assessment is based on the worst-case crop group potatoes. For Propamocarb and the formulation BAS 743 03 F, risk acceptability is indicated at Tier 1; therefore, the risk assessment is presented for all relevant crop groups. Please refer to Point 9.1.2 for further details.

The minor crops indicated in the GAP table (i.e. floriculture, avenue trees, climbing plants, conifers, ornamental shrubs, heather, forest trees and hedging plants, fruit trees and shrubs and perennial crops) are covered by the crop groups ´ornamentals´ as well as ´orchards´ and ´bush and cane fruit´ (in line with the request by the zonal Rapporteur Member State).

#### First-tier assessment (screening/generic focal species)

The results of the acute and reproductive screening risk assessments are summarised in the following tables.

Table 9.2‑8 Ametoctradin: Screening assessment of the acute and long-term/reproductive risk for birds due to the worst-case use of BAS 743 03 F in potato (3 × 2.0 L product/ha)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Potato | | | | |
| **Active substance** | | Ametoctradin | | | | |
| **Application rate (kg/ha)** | | 3 × 0.24 (min. interval: 5 days) | | | | |
| **Acute toxicity (mg/kg bw)** | | 3776 | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| Potato | Small omnivorous bird | | 158.8 | 1.79 | 68.31 | 55.3 |
| **Reprod. toxicity (mg/kg bw/d)** | | 115.2 | | | | |
| **TER criterion** | | 5 | | | | |
| **Crop scenario** | **Indicator species** | | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| Potato | Small omnivorous bird | | 64.8 | 2.21 x 0.53 | 18.36 | 6.3 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The screening assessment above shows an acceptable acute and chronic risk to birds for the active substance Ametoctradin from the proposed uses of BAS 743 03 F. No higher tier dietary risk assessments are necessary.

Table 9.2‑9 Propamocarb-HCl: Screening assessment of the acute and long-term/reproductive risk for birds due to the worst-case use of BAS 743 03 F in potato (3 × 2.0 L product/ha)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Potato | | | | |
| **Active substance** | | Propamocarb | | | | |
| **Application rate (kg/ha)** | | 3 × 0.902 | | | | |
| **Acute toxicity (mg/kg bw)** | | 3477.7 | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| Potato | Small omnivorous bird | | 158.8 | 1.79 | 256.75 | 13.5 |
| **Reprod. toxicity (mg/kg bw/d)** | | 105 | | | | |
| **TER criterion** | | 5 | | | | |
| **Crop scenario** | **Indicator species** | | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| Potato | Small omnivorous bird | | 64.8 | 2.21 x 0.53 | 68.37 | **1.5** |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The screening assessment above shows an acceptable acute risk to birds for the active substance Propamocarb-HCl from the proposed uses of BAS 743 03 F. However, a potential long-term risk to birds is indicated and therefore a first-tier long-term (reproductive) risk assessment is required.

Table 9.2‑10: First-tier assessment of the reproductive risk for birds due to the use of Propamocarb-HCl in BAS 743 03 F

| **Active substance** | Propamocarb-HCl | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **Reprod. toxicity  (mg/kg bw/d)** | 105 | | | | | |
| **TER criterion** | 5 | | | | | |
| **Growth stage** | **Generic focal species** | **App. Rate**  **(kg a.s./ha)** | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| **Potatoes (3 x 0.902 kg a.s./ha, BBCH 21-89)** | | | | | | |
| BBCH 10-39 | Small omnivorous bird “lark” | 0.902 | 10.9 | ~~1.79~~ 2.2× 0.53 | 11.43 | 9.2 |
| BBCH ≥40 | Small omnivorous bird “lark” | 3.3 | 3.46 | 30.3 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 10.17 | 10.3 |
| **Potatoes (2 x 0.902 kg a.s./ha, BBCH 21-89)** | | | | | | |
| BBCH 10-39 | Small omnivorous bird “lark” | 0.902 | 10.9 | 1.71 × 0.53 | 8.84 | 11.9 |
| BBCH ≥40 | Small omnivorous bird “lark” | 3.3 | 2.68 | 39.2 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 7.87 | 13.3 |
| **Bulbs and onion like crops (2 x 0.902 kg a.s./ha, BBCH 14–49)** | | | | | | |
| BBCH 10-39 | Small granivorous bird “finch” | 0.902 | 11.4 | 1.71 × 0.53 | 9.25 | 11.4 |
| BBCH ≥40 | Small granivorous bird “finch” | 6.9 | 5.60 | 18.8 |
| BBCH 10-39 | Small omnivorous bird “lark” | 10.9 | 8.84 | 11.9 |
| BBCH ≥40 | Small omnivorous bird “lark” | 6.5 | 5.27 | 19.9 |
| BBCH 10-19 | Small insectivorous bird “wagtail” | 11.3 | 9.17 | 11.5 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 7.87 | 13.3 |
| **Fruiting vegetables (2 x 0.902 kg a.s./ha, BBCH ~~14~~ 21- 89)** | | | | | | |
| BBCH 71-89 | Frugivorous “crow” | 0.902 | 32 | 1.62 × 0.53 | 24.56 | **4.3** |
| BBCH 10-49 | Small granivorous bird “finch” | 11.4 | 8.75 | 12.0 |
| BBCH ≥50 | Small granivorous bird “finch” | 3.4 | 2.61 | 40.2 |
| BBCH 10-49 | Small omnivorous bird “lark” | 10.9 | 8.37 | 12.5 |
| BBCH ≥50 | Small omnivorous bird “lark” | 3.3 | 2.53 | 41.5 |
| BBCH 71-89 | Frugivorous “starling” | 20.7 | 15.89 | 6.6 |
| ~~BBCH 10-19~~ | ~~Small insectivorous bird “wagtail”~~ | ~~11.3~~ | ~~8.67~~ | ~~12.1~~ |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 7.45 | 14.1 |
| **Ornamentals (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Application to plant | Small insectivorous bird “tit” | 0.902 | 18.2 | 1.62 × 0.53 | 14.76 | 7.1 |
| Application to plant - exposure to underlying ground | small insectivorous/worm feeding “thrush” | 2.7 | 2.19 | 47.9 |
| **Orchards (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Spring/Summer | Small insectivorous bird “tit” | 0.902 | 18.2 | 1.62 × 0.53 | 13.97 | 7.5 |
| Crop directed –  BBCH 10-19 | small insectivorous/worm feeding “thrush” | 2.1 | 1.61 | 65.1 |
| Crop directed –  BBCH 20-39 | small insectivorous/worm feeding “thrush” | 1.6 | 1.23 | 85.5 |
| Crop directed –  BBCH ≥ 40 | small insectivorous/worm feeding “thrush” | 0.8 | 0.61 | 171.0 |
| Crop directed –  BBCH 10-19 | Small granivorous bird “finch” | 10.1 | 7.75 | 13.5 |
| Crop directed –  BBCH 20-39 | Small granivorous bird “finch” | 7.6 | 5.83 | 18.0 |
| Crop directed –  BBCH ≥ 40 | Small granivorous bird “finch” | 3.8 | 2.92 | 36.0 |
| **Bush and cane fruit (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Whole season (currants) –  BBCH 00-79 | Small insectivorous bird “warbler” | 0.902 | 20.3 | 1.62 × 0.53 | 15.58 | 6.7 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

a Corrected SV value (to correct for known error in EFSA/2009/1438 guidance document)

The first tier risk assessment demonstrates an acceptable long-term risk for birds for the active substance Propamocarb-HCl from the use of BAS 743 03 F, except the risk to frugivorous birds “crow” from use in fruiting vegetables at BBCH 71 - 89. Therefore, further refinement is required.

Table 9.2‑11: BAS 743 03 F: Screening assessment of the acute and long-term/reproductive risk for birds due to the worst-case use of BAS 743 03 F in potato (3 × 2.0 L product/ha)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Potato | | | | |
| **Product** | | BAS 743 03 F | | | | |
| **Application rate (kg/ha)** | | 3 × ∑ (0.24 + 0.902) 1) | | | | |
| **Acute toxicity (mg/kg bw)** | | 2281.3 (measured LD50) | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| Potato | Small omnivorous bird | | 158.8 | ~~1.48~~ 1.79 | 325.06 | **7.0** |
| **Reprod. toxicity (mg/kg bw/d)** | | 107 (predicted NOELmix) | | | | |
| **TER criterion** | | 5 | | | | |
| **Crop scenario** | **Indicator species** | | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| Potato | Small omnivorous bird | | 64.8 | ~~1.71~~ 2.2 x 0.53 | 86.56 | **1.2** |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

1 Sum of active substances

The screening assessment above shows a potential acute and long-term (reproductive) risk to birds, therefore a first-tier acute and long-term risk assessment is required for the proposed uses of BAS 743 03 F.

Table 9.2‑12: BAS 743 03 F: First-tier assessment of the acute risk for birds due to the use of BAS 743 03 F at 1 – 3 x 2.0 L/ha

| **Product** | BAS 743 03 F | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **Acute toxicity  (mg/kg bw/d)** | 2281.3 (measured LD50) | | | | | |
| **TER criterion** | 10 | | | | | |
| **Growth stage** | **Generic focal species** | **App. Rate**  **(kg a.s./ha)** | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| **Potatoes (3 × ∑ (0.24 + 0.902) kg a.s./ha, BBCH 21-89)** | | | | | | |
| BBCH 10-39 | Small omnivorous bird “lark” | 1.142 | 24 | 1.79 | 49.13 | 46.4 |
| BBCH ≥40 | Small omnivorous bird “lark” | 7.2 | 14.74 | 154.8 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 25.2 | 51.58 | 44.2 |
| **Potatoes (2 × ∑ (0.24 + 0.902) kg a.s./ha, BBCH 21-89)** | | | | | | |
| BBCH 10-39 | Small omnivorous bird “lark” | 1.142 | 24 | 1.48 | 40.58 | 56.2 |
| BBCH ≥40 | Small omnivorous bird “lark” | 7.2 | 12.18 | 187.4 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 25.2 | 42.61 | 53.5 |
| **Bulbs and onion like crops (2 × ∑ 0.24 + 0.902 kg a.s./ha, BBCH 14–49)** | | | | | | |
| BBCH 10-39 | Small granivorous bird “finch” | 1.142 | 24.7 | 1.48 | 41.77 | 54.6 |
| BBCH ≥40 | Small granivorous bird “finch” | 14.8 | 25.03 | 91.2 |
| BBCH 10-39 | Small omnivorous bird “lark” | 24 | 40.58 | 56.2 |
| BBCH ≥40 | Small omnivorous bird “lark” | 14.4 | 24.35 | 93.7 |
| BBCH 10-19 | Small insectivorous bird “wagtail” | 26.8 | 45.32 | 50.3 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 25.2 | 42.61 | 53.5 |
| ~~BBCH ≥20~~ | ~~Small insectivorous bird “wagtail”~~ | ~~25.2~~ | ~~41.77~~ | ~~54.6~~ |
| **Fruiting vegetables (2 × ∑ 0.24 + 0.902 kg a.s./ha, BBCH ~~14~~ 21 - 89)** | | | | | | |
| BBCH 71-89 | Frugivorous “crow” | 1.142 | 57.4 | 1.41 | 92.33 | 24.7 |
| BBCH 10-49 | Small granivorous bird “finch” | 24.7 | 39.73 | 57.4 |
| BBCH ≥50 | Small granivorous bird “finch” | 7.4 | 11.90 | 191.7 |
| BBCH 10-49 | Small omnivorous bird “lark” | 24 | 38.60 | 59.1 |
| BBCH ≥50 | Small omnivorous bird “lark” | 7.2 | 11.58 | 197.0 |
| BBCH 71-89 | Frugivorous “starling” | 49.4 | 79.46 | 28.7 |
| ~~BBCH 10-19~~ | ~~Small insectivorous bird “wagtail”~~ | ~~26.8~~ | ~~43.11~~ | 52.9 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 25.2 | 40.53 | 56.3 |
| **Ornamentals (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Application to plant | Small insectivorous bird “tit” | 1.142 | 18.2 | 1.41 | 75.28 | 30.3 |
| Application to plant - exposure to underlying ground | small insectivorous/worm feeding “thrush” | 2.7 | 11.90 | 191.7 |
| **Orchards (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Spring/Summer | Small insectivorous bird “tit” | 1.142 | 46.8 | 1.41 | 75.28 | 30.3 |
| Crop directed –  BBCH 10-19 | small insectivorous/worm feeding “thrush” | 5.9 | 9.49 | 240.4 |
| Crop directed –  BBCH 20-39 | small insectivorous/worm feeding “thrush” | 4.4 | 7.08 | 322.3 |
| Crop directed –  BBCH ≥ 40 | small insectivorous/worm feeding “thrush” | 2.2 | 3.54 | 644.7 |
| Crop directed –  BBCH 10-19 | Small granivorous bird “finch” | 21.9 | 35.23 | 64.8 |
| Crop directed –  BBCH 20-39 | Small granivorous bird “finch” | 16.4 | 26.38 | 86.5 |
| Crop directed – BBCH ≥ 40 | Small granivorous bird “finch” | 8.2 | 13.19 | 173.0 |
| **Bush and cane fruit (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Whole season (currants) –  BBCH 00-79 | Small insectivorous bird “warbler” | 1.142 | 52.2 | 1.41 | 83.96 | 27.2 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

a Corrected SV value (to correct for known error in EFSA/2009/1438 guidance document)

Table 9.2‑13: BAS 743 03 F: First-tier assessment of the reproductive risk for birds due to the use of BAS 743 03 F at 1 – 3 x 2.0 L/ha

| **Product** | BAS 743 03 F | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **Reprod. toxicity  (mg/kg bw/d)** | 107 (predicted NOELmix) | | | | | |
| **TER criterion** | 5 | | | | | |
| **Growth stage** | **Generic focal species** | **App. Rate**  **(kg a.s./ha)** | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| **Potatoes (3 × ∑ (0.24 + 0.902) kg a.s./ha, BBCH 21-89)** | | | | | | |
| BBCH 10-39 | Small omnivorous bird “lark” | 1.142 | 10.9 | 2.21 × 0.53 | 14.47 | 7.4 |
| BBCH ≥40 | Small omnivorous bird “lark” | 3.3 | 4.38 | 24.4 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 12.88 | 8.3 |
| **Potatoes (2 × ∑ (0.24 + 0.902) kg a.s./ha, BBCH 21-89)** | | | | | | |
| BBCH 10-39 | Small omnivorous bird “lark” | 1.142 | 10.9 | 1.71 × 0.53 | 11.19 | 9.6 |
| BBCH ≥40 | Small omnivorous bird “lark” | 3.3 | 3.39 | 31.6 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 9.96 | 10.7 |
| **Bulbs and onion like crops (2 × ∑ 0.24 + 0.902 kg a.s./ha, BBCH 14–49)** | | | | | | |
| BBCH 10-39 | Small granivorous bird “finch” | 1.142 | 11.4 | 1.71 × 0.53 | 11.71 | 9.1 |
| BBCH ≥40 | Small granivorous bird “finch” | 6.9 | 7.09 | 15.1 |
| BBCH 10-39 | Small omnivorous bird “lark” | 10.9 | 11.19 | 9.6 |
| BBCH ≥40 | Small omnivorous bird “lark” | 6.5 | 6.67 | 16.0 |
| BBCH 10-19 | Small insectivorous bird “wagtail” | 11.3 | 11.60 | 9.2 |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 9.96 | 10.7 |
| **Fruiting vegetables (2 × ∑ 0.24 + 0.902 kg a.s./ha, BBCH ~~14~~ 21 - 89)** | | | | | | |
| BBCH 71-89 | Frugivorous “crow” | 1.142 | 32 | 1.62 × 0.53 | 31.10 | **3.4** |
| BBCH 10-49 | Small granivorous bird “finch” | 11.4 | 11.08 | 9.7 |
| BBCH ≥50 | Small granivorous bird “finch” | 3.4 | 3.30 | 32.4 |
| BBCH 10-49 | Small omnivorous bird “lark” | 10.9 | 10.59 | 10.1 |
| BBCH ≥50 | Small omnivorous bird “lark” | 3.3 | 3.21 | 33.4 |
| BBCH 71-89 | Frugivorous “starling” | 20.7 | 20.12 | 5.3 |
| ~~BBCH 10-19~~ | ~~Small insectivorous bird “wagtail~~” | ~~11.3~~ | ~~10.98~~ | ~~9.7~~ |
| BBCH ≥20 | Small insectivorous bird “wagtail” | 9.7 | 9.43 | 11.4 |
| **Ornamentals (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Application to plant | Small insectivorous bird “tit” | ~~0.902~~ 1.142 | 18.2 | 1.62 × 0.53 | 17.69 | 6.0 |
| Application to plant - exposure to underlying ground | small insectivorous/worm feeding “thrush” | 2.7 | 2.62 | 40.8 |
| **Orchards (2 x ∑ 0.24 + 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Spring/Summer | Small insectivorous bird “tit” | 1.142 | 18.2 | 1.62 × 0.53 | 17.69 | 6.0 |
| Crop directed –  BBCH 10-19 | small insectivorous/worm feeding “thrush” | 2.1 | 2.04 | 52.4 |
| Crop directed –  BBCH 20-39 | small insectivorous/worm feeding “thrush” | 1.6 | 1.55 | 68.8 |
| Crop directed –  BBCH ≥ 40 | small insectivorous/worm feeding “thrush” | 0.8 | 0.78 | 137.6 |
| Crop directed –  BBCH 10-19 | Small granivorous bird “finch” | 10.1 | 9.82 | 10.9 |
| Crop directed –  BBCH 20-39 | Small granivorous bird “finch” | 7.6 | 7.39 | 14.5 |
| Crop directed –  BBCH ≥ 40 | Small granivorous bird “finch” | 3.8 | 3.69 | 29.0 |
| **Bush and cane fruit (2 x ∑ 0.24 + 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Whole season (currants) –  BBCH 00-79 | Small insectivorous bird “warbler” | 1.142 | 20.3 | 1.62 × 0.53 | 19.73 | 5.4 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

a Corrected SV value (to correct for known error in EFSA/2009/1438 guidance document)

The first tier risk assessment demonstrates an acceptable acute and long-term risk for birds for BAS 743 03 F, except the chronic risk to frugivorous birds “crow” from use in fruiting vegetables at BBCH 71 – 89. Therefore, further refinement is required.

#### Higher-tier risk assessment

According to the above first-tier risk assessment, a long-term higher-tier risk assessments is required for Propamocarb-HCl and BAS 743 03 F and the frugivorous birds “crow” from the use in fruiting vegetables at BBCH 71 – 89.

**Frugivorous birds “crow”**

The generic focal species as defined in EFSA/2009/1438, can be refined in a higher tier risk assessment by defining a realistic focal species that actually occurs in the crop and in the geographic region based on crop-specific generic bird data.

The generic focal species starling and crow both reflect the risk to frugivorous birds at BBCH 71-89, while the Tier 1 assessment assumes a diet consisting of 100% fruits for both species with the highest RUD value in gourds of 34.3 mg/kg. Therefore, the Tier 1 scenario frugivorous bird “crow” represents an unrealistic worst -case for the use of BAS 743 03 F in tomatoes and aubergine. Accordingly, the risk to frugivorous birds is considered to be covered by the scenario frugivorous birds “starling” with a RUD value for tomatoes of 20.7 mg/kg, for which an acceptable risk was demonstrated in the first-tier risk assessment.

However, as a conservative approach a refined risk assessment for frugivorous birds “crow” is presented below considering refined exposure and a refined portion of diet (PD).

The occurrence of birds in fruiting vegetables (tomatoes) has been evaluated in a generic field study conducted in commercially used tomato fields (3.7 - 7 ha) in the Southern Zone (Codogno, Lombardia, Italy) For details please refer to the RAR for Propamocarb, Vol. 3 CP, Proplant, B9 (June 2017). The study area is a typical region for the cultivation of field tomatoes in southern Europe. Bird activities and abundance in the tomato fields were recorded on two whole days in each field by an ornithologist and every bird in the field was recorded (species, numbers, behaviour). Additionally, three times within the study period a bird census was carried out at each study field and its vicinity, to get a list of species and their relative abundance in tomato growing areas and surrounding habitats. Tomato fields are intensively cultivated areas. The fields were covered by plants to fifty percent approximately. Only a small proportion of the plants were weeds while the majority were cultivated tomatoes.

The study showed that the abundance and diversity of birds in the tomato fields were very low, compared to the surrounding habitats. Main species on the fields were the Yellow Wagtail, even breeding on the fields, but strictly insectivore, and the Tree- and House Sparrow, both omnivorous but obviously exclusively feeding on insects within this habitat. The only species, observed feeding on vegetable material, was the Hooded Crow, pecking on the inner parts of tomato fruits.

Fruiting vegetables, such as tomatoes and aubergine, are grown and managed in the Central Zone by various techniques in open fields or greenhouses. The fruiting vegetables are usually grown in rows as it allows for better management of the crop, easier access for harvesting and irrigation. The direct area under the crop is usually kept free of vegetation to avoid competition of the plants while the area between the rows is usually covered by vegetation. Based on this, in the Central Zone, the field structure and farming practices are comparable for the different types of fruiting vegetables. Therefore, the findings on the occurrence of birds in tomato fields obtained from the above generic field study is considered suitable to refine the long-term/reproductive risk to frugivorous birds from the use of BAS 743 03 F in fruiting vegetables.

The hooded crow (*Corvus cornix*) was the only bird species observed feeding on vegetable material, while pecking on the inner parts of tomato fruits. Therefore, the hooded crow (*Corvus Cornix*) is considered the relevant focal species obtaining parts of its diet in fruiting vegetable fields instead of the American crow (*Corvus brachyrhynchos*) which is listed in EFSA/2009/1438 as a generic focal species for frugivorous birds in fruiting vegetables. In addition, for most European regions (Southern European and Central European), the carrion crow (*Corvus corone*), the rook (Corvus frugilegus) and the hooded crow (*Corvus cornix*) are considered to be of higher relevance (Cramp et al., 1998; Glutz von Blotzheim & Bauer, 2001), all of which are omnivorous bird species.

In the Tier-1 risk assessment it is assumed that the generic focal species “crow” feeds exclusively on fruits in the treated field. However, this is an unrealistic assumption considering the feeding behaviour of the crow as an omnivorous bird. Buxton et al. (1998)[[1]](#footnote-1) describe a series of studies which have investigated the diet composition of different crow species *i.e*., the carrion crow, rook and hooded crow. Results show that the diet of these crow species consists mainly of insects, earthworms, fruits, plant matter (incl. cereals, plant matter and grain), and carrion.

In particular for the hooded crow, the diet as percentages of volume found in stomachs during a period between April and September consists of 70% insects, 5% earthworms, 5% fruits and 15% plant material. For the period October to March, the fractions in the diet are 70% cereal grain, 15% plants, 5% carrion and 5% fruits (Houston, 1977, in Buxton et al., 1998).

Although these observations on dietary compositions were made in the Central Zone (UK) and not specifically in vegetable fields, it is highly unlikely that crows in fruiting vegetables feed exclusively on fruits (*i.e*. 100%). Therefore, it can be conservatively assumed, based on feeding habits of omnivores that the diet of crows in fruiting vegetables consists of various food items other than fruits. Accordingly, also considering the fact that in the generic field study most birds observed in the field were feeding exclusively on insects, it is likely that a significant proportion of insects in the diet will rather be obtained from the plants or from soil surface with distinctly lower RUDs.

Therefore, a refined higher tier risk assessment is shown below assuming a more realistic worst-case proportion of diet (PD value) of 70% insects (worst-case RUD for foliar insects of 21 mg a.s./kg) and 30% fruits (worst-case RUD for gourds of 34.3 mg a.s./kg). This is a conservative assumption as data (Houston, 1977, in Buxton et al., 1998) suggest that insects with a lower RUD are dominant in the diet of crows, whereas fruits are taken at distinctly lower percentages. Also for the other feed items reported in the diet of omnivorous crows, specifically for earthworms, carrion or non-grass herbs, residue levels can be assumed lower as the RUD for gourds.

Furthermore, the generic field study showed that while some birds visited the fields only sporadically or singularly (7 out of 10 observations), most birds were observed in the surrounding areas of the field (38 species, 95% compared to 5% in tomato fields). From these birds visiting the fields, only 49 observations of feeding behaviour on tomato fields were made. These were mainly birds feeding on insects (36 in total, 73.5%), 3 unknown items and 10 times (20.4%) the hooded crow picking the fruits. Based on these results, it can be assumed that crows will not feed exclusively on the treated fruits in the field but prefer a mixed or even exclusively animal diet which they will mostly obtain in the untreated surroundings of the field. In addition, the assumption that crows will be able to feed exclusively on fruits in economically managed fields is highly unrealistic on a long-term view. Moreover, growers will take measures to minimise damage to fruits by birds, for example by using netting. The potential for consumption of fruits (*i.e*. the crop itself) is therefore minimised. Considering all this, it is highly likely that a PT value (the time spent feeding in the treated field) of 1 overestimate realistic exposure of birds in tomato fields. Therefore, as a realistic worst-case a PT value of 0.8 was used in the refined risk assessment below.

|  |
| --- |
| **Review Comments:**  The information and data provided above by the Applicant, in the opinion of zRMS, can only be used as supplementary data. |

**Residue per Unit Doses (RUD)**

In order to refine the assessments based on the proposed generic focal species, reference is made to RUD data as provided in the updated Birds and Mammals Guidance (EFSA, 2023[[2]](#footnote-2)). It is acknowledged that this guidance document is not yet noted. However, the revised residue data are in agreement with scientific developments (favouring geometric mean residue estimates over arithmetic means) and are therefore argued to be more relevant, independent of an implementation of the revised guidance.

The geometric mean RUDs for cucurbitaceous plants (fruits) according to the revised RUD data is 0.47 mg a.s./kg.

Revised risk assessments independent of additional refinements as detailed above for the “crow” are presented below based on these RUD estimates.

|  |
| --- |
| **Review Comments:**  In the updated Birds and Mammals Guidance (EFSA, 2023) tomatoes were included in the crop group ‘fruiting solanaceous vegetable crops’  The default RUD values given in EFSA (2009) had several shortcomings (e.g., only few data were available for some matrices). Nowadays available information on residues in ‘fruiting solanaceous vegetable crops’ has been evaluated according to current standards and using a larger dataset (the database developed by Lahr et al.; 2018). For this crop group data were available from 97 samples, where for tomatoes - 88% and peppers – 12%. In z RMS opinion, the residue data evaluated for the purposes of revision B&M guidance are the most reliable for the regulatory use. The WG considered that a geometric mean value was preferable to the arithmetic mean which was used in EFSA (2009) as there is less influence of the extreme values in the distribution.  The geometric mean RUDs for ‘fruiting solanaceous vegetable crops’ according to the revised RUD data is 0.73 mg a.s./kg (Table J.8).  Only for tomatoes the exposure was refined using RUDm value from EFSA (2023). For other exposure scenarios it was not required as acceptable risk was concluded using more conservative RUD values from EFSA (2009). |

**Refined risk assessments**

In line with the risk assessments failing at Tier 1, below revised risk assessments are presented for propamocarb-HCl and the formulated product BAS 743 03 F (based on NOELmix) for the frugivorous “crow”.

Table 9.2‑14: Higher-tier assessment of the long-term/reproductive risk for the frugivorous bird “crow” due to the exposure to propamocarb-HCl following the uses of BAS 743 03 F in fruiting vegetables (BBCH 71 – 89) – revised PD, PT

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Reprod. toxicity (mg/kg bw/d)** | | | 105 | | | | | | | |
| **App. Rate (kg a.s./ha)** | | | 2 x 0.902 | | | | | | | |
| **TER criterion** | | | 5 | | | | | | | |
| **Max. application rate (g a.s./ha)** | **Feed item** | **PD** | **FIRi, total fresh**  **[g fresh weight/d]** | **RUD of food item**  **[mg a.s./kg]** | **MAF × twa\*** | **DF** | **PT** | **DDDm**  **(mg/kg bw/d)** | **Body weight [g]** | **TERlt** |
| **Fruiting vegetables** | | | | | | | | | | |
| 2 x 902  (7 d interval) | Foliar insects | 0.7 | 157.11 | **21** | 0.95 | 1 | **0.8** | 6.01 | 448 | 17.5 |
| Fruits | 0.3 | **34.3** |

FIR: food intake rate; TWA: time-weighted average factor; DDD: daily dietary dose; DF: Deposition factor, PD: Fraction in diet, PT: Fraction in time, TER: toxicity to exposure ratio. **Bold**: refined parameter(s)

\*MAF×twa value for Propamocarb-HCl calculated with the moving-time window approach for 2 applications and 7-day interval according to the formula detailed in Appendix H of the EFSA Guidance Document (2009).

Table 9.2‑15: Higher-tier assessment of the long-term/reproductive risk for the frugivorous bird “crow” due to the exposure to propamocarb-HCl following the uses of BAS 743 03 F in fruiting vegetables (BBCH 71 – 89) – revised RUD

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Reprod. toxicity (mg/kg bw/d)** | | | 105 | | | | | | | |
| **App. Rate (kg a.s./ha)** | | | 2 x 0.902 | | | | | | | |
| **TER criterion** | | | 5 | | | | | | | |
| **Max. application rate (g a.s./ha)** | **Feed item** | **PD** | **FIRi, total fresh**  **[g fresh weight/d]** | **RUD of food item**  **[mg a.s./kg]** | **MAF × twa\*** | **DF** | **PT** | **DDDm**  **(mg/kg bw/d)** | **Body weight [g]** | **TERlt** |
| **Fruiting vegetables** | | | | | | | | | | |
| 2 x 902  (7 d interval) | Fruits | 1.0 | 417.93 | **~~0.47~~ 0.73** | 0.95 | 1 | 1 | ~~0.38~~ 0.58 | 448 | ~~279.5~~  181 |

FIR: food intake rate; TWA: time-weighted average factor; DDD: daily dietary dose; DF: Deposition factor, PD: Fraction in diet, PT: Fraction in time, TER: toxicity to exposure ratio. **Bold**: refined parameter(s)

\*MAF×twa value for Propamocarb-HCl calculated with the moving-time window approach for 2 applications and 7-day interval according to the formula detailed in Appendix H of the EFSA Guidance Document (2009).

Table 9.2‑16: Higher-tier assessment of the long-term/reproductive risk for the frugivorous bird “crow” due to the exposure to BAS 743 03 F in fruiting vegetables (BBCH 71 – 89) – revised PD, PT

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Reprod. toxicity (mg/kg bw/d)** | | | 107 (NOELmix) | | | | | | | |
| **App. Rate (kg a.s./ha)** | | | 2 x 1.142 (2 × ∑ 0.24 + 0.902) | | | | | | | |
| **TER criterion** | | | 5 | | | | | | | |
| **Max. application rate (g a.s./ha)** | **Feed item** | **PD** | **FIRi, total fresh**  **[g fresh weight/d]** | **RUD of food item**  **[mg a.s./kg]** | **MAF × twa\*** | **DF** | **PT** | **DDDm**  **(mg/kg bw/d)** | **Body weight [g]** | **TERlt** |
| **Fruiting vegetables** | | | | | | | | | | |
| 2 x 902  (7 d interval) | Foliar insects | 0.7 | 157.11 | **21** | 0.95 | 1 | **0.8** | 7.61 | 448 | 14.1 |
| Fruits | 0.3 | **34.3** |

FIR: food intake rate; TWA: time-weighted average factor; DDD: daily dietary dose; DF: Deposition factor, PD: Fraction in diet, PT: Fraction in time, TER: toxicity to exposure ratio. **Bold**: refined parameter(s)

\*MAF×twa value for Propamocarb-HCl calculated with the moving-time window approach for 2 applications and 7-day interval according to the formula detailed in Appendix H of the EFSA Guidance Document (2009).

Table 9.2‑17: Higher-tier assessment of the long-term/reproductive risk for the frugivorous bird “crow” due to the exposure to BAS 743 03 F in fruiting vegetables (BBCH 71 – 89) – revised RUD

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Reprod. toxicity (mg/kg bw/d)** | | | 107 (NOELmix) | | | | | | | |
| **App. Rate (kg a.s./ha)** | | | 2 x 1.142 (2 × ∑ 0.24 + 0.902) | | | | | | | |
| **TER criterion** | | | 5 | | | | | | | |
| **Max. application rate (g a.s./ha)** | **Feed item** | **PD** | **FIRi, total fresh**  **[g fresh weight/d]** | **RUD of food item**  **[mg a.s./kg]** | **MAF × twa\*** | **DF** | **PT** | **DDDm**  **(mg/kg bw/d)** | **Body weight [g]** | **TERlt** |
| **Fruiting vegetables** | | | | | | | | | | |
| 2 x 902  (7 d interval) | Fruits | 1.0 | 417.93 | **~~0.47~~ 0.73** | 0.95 | 1 | 1 | ~~0.48~~ 0.74 | 448 | ~~224.9~~  144.6 |

FIR: food intake rate; TWA: time-weighted average factor; DDD: daily dietary dose; DF: Deposition factor, PD: Fraction in diet, PT: Fraction in time, TER: toxicity to exposure ratio. **Bold**: refined parameter(s)

\*MAF×twa value for Propamocarb-HCl calculated with the moving-time window approach for 2 applications and 7-day interval according to the formula detailed in Appendix H of the EFSA Guidance Document (2009).

The refined risk assessments for frugivorous birds feeding in fruiting vegetables following application of BAS 743 03 F demonstrate acceptable risk.

#### Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf*. Appendix K of EFSA/2009/1438).

Leaf scenario

Since BAS 743 03 F is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)OC of 3779 L/kg and of 535.56 L/kg for Ametoctradin and Propamocarb, respectively both active substances belong to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group potatoes also covers the risk for birds from all other intended uses in fruiting vegetables and bulbs and onion like crops. The ratio calculations for effective application rate to relevant endpoints are detailed in Table 9.2‑18 and Table 9.2‑19.The ratios for acute and reproductive endpoints for Ametoctradin (0.15 and 5.1, respectively) do not exceed the threshold values of 3000, thus no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary. Therefore, a quantitative drinking water risk assessment for the puddle scenario is not triggered.

Table 9.2‑18: Assessment of the risk for birds due to exposure to Ametoctradin via contaminated drinking water in puddles

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Ametoctradin** | **Reference** |
| Koc (geometric mean) [L/kg] | 3779 | Chapter 8.5.1 and 8.5.2 |
| Number of applications | 3 | See above |
| Interval [days] | 5 | See above |
| MAFm 1) | 2.21 | -- |
| Max use rate [g/ha] | 240 | See above |
| AReff [g/ha] 2) | 530 | -- |
| LD50 [mg/kg b.w.] | 3776 | See above |
| Ratio (acute) 3) | 0.1403 | -- |
| NO(A)EL [mg/kg b.w./d] | 115.2 | See above |
| Ratio (repro) 3) | 4.5981 | -- |
| Trigger | 3000 | -- |
| Drinking water assessment  required [Yes/No] | No | -- |

1) MAFm = (1-e-nki) / (1-e-ki) with k = ln(2)/DT50 (rate constant), n = number of applications and i = application interval [d]

2) AReff = Application rate (g/ha) x MAFmean

3) Ratio of AReff and relevant toxicity endpoint

Table 9.2‑19: Assessment of the risk for birds due to exposure to Propamocarb-HCl via contaminated drinking water in puddles

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Propamocarb-HCl** | **Reference** |
| Koc (geometric mean) [L/kg] | 535.56 | Chapter 8.5.1 and 8.5.2 |
| DT50 (soil) [days] | 10.19 | Chapter 8.3.1.1 and 8.3.1.2 |
| Number of applications | 3 | See above |
| Interval [days] | 5 | See above |
| MAFm 1) | 2.21 | -- |
| Max use rate [g/ha] | 902 | See above |
| AReff [g/ha] 2) | 1991 | -- |
| LD50 [mg/kg b.w.] | 3477.7 | See above |
| Ratio (acute) 3) | 0.5725 | -- |
| NO(A)EL [mg/kg b.w./d] | 105 | See above |
| Ratio (repro) 3) | 18.9601 | -- |
| Trigger | 3000 | -- |
| Drinking water assessment  required [Yes/No] | No | -- |

1) MAFm = (1-e-nki) / (1-e-ki) with k = ln(2)/DT50 (rate constant), n = number of applications and i = application interval [d]

2) AReff = Application rate (g/ha) x MAFmean

3) Ratio of AReff and relevant toxicity endpoint

In conclusion, the risk to birds via drinking water from the intended use of BAS 743 03 F according to the proposed use pattern is acceptable.

#### Effects of secondary poisoning

According to the EFSA/2009/1438, substances with a log Pow ≥ 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains.

The log Pow of the active substance Ametoctradin is 4.4 (XXXX DocID 2005/1014072), which triggers an assessment of the potential risk from secondary poisoning. The log Pow of the active substance Propamocarb is -1.2 (at pH 7, EFSA Scientific Report 2006, 78, 1-80), which does not trigger an assessment of the potential risk from secondary poisoning.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil is based on experimental data.

As shown in the following table, the TERLT for Ametoctradin exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning.

Table ‑20: Assessment of the risk for earthworm-eating birds due to exposure to Ametoctradin via bioaccumulation in earthworms (secondary poisoning) for the worst-case intended use in onions ~~ornamentals (perennial crops)~~

| **Parameter** | Ametoctradin | **Reference** |
| --- | --- | --- |
| PECsoil (twa, 21 days)[mg/kg soil] 1) | ~~0.135~~ 0.145 | Chapter 8.7 (Table 8.7-6) |
| Kow | 25119 | XXXXX DocID 2005/1014072 |
| Koc (arithmetic mean) | 3779 | EFSA Journal 2012, 10(11): 2921;  XXXXX DocID 2008/1017000 and 2008/1046556 |
| foc (default) | 0.02 | EFSA/2009/1438 |
| BCFearthworm | 4.013 | -- |
| PECworm [mg/kg] | ~~0.542~~ 0.582 | PECworm = PECsoil x BCFearthworm |
| Daily dose [mg/kg b.w./d] | ~~0.569~~ 0.611 | Daily dose = PECworm x 1.05 |
| NO(A)EL [mg/kg b.w./d] | 115.2 | See above |
| TERlt | ~~202.5~~ 188.5 | -- |

1) Highest PECsoil (21-d, twa) as worst-case, selected from 2 application in onion ~~ornamentals (perennial crops)~~ as a risk envelope.

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

As shown in the following table, the TERLT for Ametoctradin exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating birds via secondary poisoning.

Table ‑21: Assessment of the risk for fish-eating birds due to exposure to Ametoctradin via bioaccumulation in fish (secondary poisoning) for the worst-case intended use in potato

| **Parameter** | **Ametoctradin** | **Reference** |
| --- | --- | --- |
| PECsw [mg/L] 1) | 0.1645 | Chapter 8.9 (Step 1) |
| BCF fish (max. worst case) | 219 | EFSA Journal 2012, 10(11): 2921 |
| PECfish [mg/kg] | 36.026 | PECfish = PECwater x TWA x BCF |
| Daily dose [mg/kg b.w./d] | 5.728 | Daily dose = PECfish x 0.159 |
| NO(A)EL [mg/kg b.w./d] | 115.2 | See above |
| TERlt | 20.1 | -- |

1) Highest PECsw (initial) as worst-case, selected from single application scenario in potatoes, onion and salad crops (spray drift). For details please refer to chapter 8.9.

#### Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU reviews of Ametoctradin (EFSA Scientific Report (2012) 10(11): 2921) and Propamocarb-HCl (EFSA Scientific Report (2006)) from toxicokinetics studies.

Since the bioaccumulation potential of Ametoctradin and Propamocarb is low, no further assessment on biomagnification along the food chain is required.

### Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

### Overall conclusions

It can be concluded that the risk to birds from the application of BAS 743 03 F according to good agricultural practice is acceptable.

|  |
| --- |
| **Review Comments:**  The acute and chronic risks of BAS 743 03 Fto birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients, and maximum residues occurring on food items.  All TER values exceed the relevant triggers indicating that BAS 743 03 Fdoes not pose an unacceptable risk to birds following applications according to recommended use pattern.  Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The risk to earthworm- and fish-eating animals from secondary poisoning is low. |

## Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

### Toxicity data

Mammalian toxicity studies have been carried out with Ametoctradin and Propamocarb. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on mammals of BAS 743 03 F were not evaluated as part of the EU assessment of the active substances Ametoctradin and Propamocarb. A new acute oral study on the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) with rats submitted with this application is listed in Appendix 1 and summarised in Appendix 2. Since differences in co-formulants and/or their concentration between both formulations are considered minimal and both formulations are SC (suspension concentrates), it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission. In addition, several ecotoxicological bridging studies have been performed with the formulation BAS 743 03 F on aquatic invertebrates (*Daphnia magna*, BAS Doc ID 2022/2033730), adult honey bees (*Apis mellifera*, acute oral and contact, XXXX Doc ID 2022/2033729), non-target terrestrial arthropods (*Aphidius rhopalosiphi,* XXXX Doc ID 2022/2033732) and chronic earthworms (*Eisenia fetida*, XXXX Doc ID 2022/2033731) indicating no increased toxicity of BAS 743 03 F compared to BAS 743 02 F based on the content of active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process, plus the endpoint for the formulation has been taken into account.

**Active substances**

An overview of the EU agreed endpoints for Ametoctradin is given in . In case the selection of studies and endpoints for the risk assessment deviates from the results of the EU review process, justifications are provided below.

Table ‑: Ametoctradin (BAS 650 F): Endpoints relevant for the risk assessment for mammals

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| Rat | Ametoctradin | Oral 1 d Acute | LD50 > 2000 mg/kg bw | EFSA Journal 2012, 10(11): 2921;  2006/1025795 |
| Rat | Ametoctradin | Dietary Reproductive toxicity Two-generation study | NOAELparental = 939 mg a.s./kg bw/d  NOAELreproductive = 939 mg a.s./kg bw/d  NOAELoffspring = 939 mg a.s./kg bw/d | EFSA Journal 2012, 10(11): 2921; 2008/1014201 |
| Rat | Ametoctradin | Oral Developmental toxicity | NOAELmaternal = 1000 mg a.s./kg bw/d  NOAELdevelopmental = 1000 mg a.s./kg bw/d | EFSA Journal 2012, 10(11): 2921; 2006/1024669 |
| Rabbit | Ametoctradin | Oral Developmental toxicity | NOAELmaternal = 1000 mg a.s./kg bw/d  NOAELdevelopmental = 1000 mg a.s./kg bw/d | EFSA Journal 2012, 10(11): 2921; 2008/1033420 |
| **Endpoint used for acute risk assessments** | **Ametoctradin** | **Oral 1 d Acute** | **LD50 > 2000 mg/kg b.w. 2)** | **EFSA Journal 2012, 10(11): 2921;**  **2006/1025795** |
| **Endpoint used for reproductive risk assessments** | **Ametoctradin** | **Dietary Reproductive toxicity Two-generation study** | **NOEL = 939 mg a.s./kg b.w./d** | **EFSA Journal 2012, 10(11): 2921;** |

1. Amendment of report XXXX DocID 2010/1031186
2. New endpoint. For details see section 9.3.1.1.

Table ‑2: Propamocarb-HCl: Endpoints relevant for the risk assessment for mammals

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| Rat | Propamocarb-HCl | Oral 1 d Acute | **~~LD~~~~50~~ ~~= 2000 mg/kg bw\*~~**  **LD50 >**  **1330 mg a.s./kg bw** | ~~RAR Propamocarb, Vol. 3 – CP B9 (June 2017)~~  EFSA Scientific Report 2006, 78, 1-80 |
| Rat | Propamocarb-HCl | Dietary Reproductive toxicity Two-generation study | **NOAELreproductive = 104 mg a.s./kg bw/d** | EFSA Scientific Report 2006, 78, 1-80 |

\* ~~According to the RAR (2017) for Propamocarb (RAR Propamocarb, Vol. 3 – CP B9, June 2017), dose-response study where rats were used with 6 doses of Previcur N covering a range of 1300 – 4826 mg/kg bw – Previcur N contained 66.5% of Propamocarb- HCl (SN 66752); The LD~~~~50~~ ~~values in this study are 2900 mg/kg bw in males and 2000 mg/kg bw in females. In the past, however, these values were erroneously corrected with a purity factor of 66.5 %, resulting in LD~~~~50~~ ~~values of 1928.5 and 1330 mg/kg bw for males and females, respectively leading to determine an LD~~~~50~~ ~~value higher than 1330 mg/kg bw used in the risk assessment. As stated in the report, the rat LD~~~~50~~ ~~values represent dose levels as active ingredient, so that the LD~~~~50~~ ~~values to be used for ecotoxicology are 2900 and 2000 mg/kg bw for males and females, respectively; a geometric mean cannot be used because there is a clear indication of a difference in sensitivity between sexes (refer EFSA Guidance Document 2009). Therefore, as a worst-case the LD~~~~50~~ ~~= 2000 mg/kg bw for females is proposed for the risk assessment.~~

**Metabolites**

See Section 9.2.1 in the bird chapter.

**Mixture toxicity**

An acute oral mammalian toxicity test with the similar formulation BAS 74302 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) is available indicating that the product is non-toxic by oral route in rats, *i.e.* LD50 > 2000 mg product/kg bw.

Table ‑3: Formulation BAS 743 03 F: Endpoints relevant for the risk assessment for mammals

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| Rat | BAS 743 02 F | Oral 1 d Acute | LD50 > 2000 mg/kg bw | New study  2022/2034982 |

\* Study was conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl

Nevertheless, in line with EFSA/2009/1438, for the assessment of acute effects (mortality) from the simultaneous exposure of mammals to residues of Propamocarb and Ametoctradin (considered to be the only potential toxicants contained in the formulation) a surrogate LD50 (mix) is additionally derived. The surrogate LD50 (mix) is calculated assuming dose additivity of toxicity by using the following equation:



With:

X (a.s.i) = fraction of active substance [i] in the mixture

LD50 (a.s.i) = acute toxicity value for active substance [i] (pragmatically, NOEL (a.s.i) may be inserted, too)

In addition to acute, the long-term risk from combined exposure of birds and mammals to active substances needs to be addressed via the Concentration Addition Model.

The estimation of the LD50 (mix) and NOEL (mix) is shown in the following tables**.**

Table ‑4: Estimation of LD50 for the mixture assuming dose additivity

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Active substance** | **Concentration of each active substance in ~~formulation~~ BAS 743 02 F**  **[g a.s.i/L]** | **X (a.s.i) in the mixture** | **LD50 [mg a.s.i/kg bw]** | **~~Σ [X (a.s.~~~~i~~~~)/LD~~~~50~~ ~~(a.s.~~~~i~~~~)]~~** | **LD50 (mix) [Σ mg a.s.i/kg bw]** |
| Ametoctradin | 137 | 0.21 | >2000 | ~~< 5.00E-04~~ | > ~~2000~~ 1430.7 |
| Propamocarb-HCl | 515 1) | 0.79 | > ~~2000~~ 1330 1) |

1) Expressed as Propamocarb-Hydrochloride

Table ‑5: Estimation of NOEL for the mixture assuming dose additivity

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Active substance** | **Concentration of each active substance in ~~formulation~~ BAS 743 02 F**  **[g a.s.i/L]** | **X (a.s.i) in the mixture** | **NOEL [mg a.s.i/kg bw]** | **Σ [X (a.s.i)/NOEL (a.s.i)]** | **NOEL (mix) [Σ mg a.s.i/kg bw]** |
| Ametoctradin | 137 | 0.21 | 939 | 7.82E-03 | 127.88 |
| Propamocarb-HCl | 515 1) | 0.79 | 104 1) |

1) Expressed as Propamocarb-Hydrochloride

Measured LD50 values, if available, should only be replaced in the risk assessment by modelled data if a significant change of the predicted risk is to be expected. To achieve a basis for a comparison of single active substance and mixture toxicity in terms of potential risk, a “tox per fraction” quotient can be calculated for each active substance and compared to the corresponding quotient for the mixture:





Table ‑6: Comparison of “tox per fraction (a.s.i)” and “tox per fraction (mix)” for acute toxicity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Active substance** | **LD50 (a.s.i) [mg a.s./kg bw]** | **X (a.s.i) in the mixture** | **Tox per fraction:**  **[LD50 (a.s.i)/X (a.s.i)]** | **Contribution to overall toxicity  [%] 1)** |
| LD50 (mix) | > ~~2000~~ 1430.7 | 1.0 | > ~~2000~~ 1430.7 | - |
| Ametoctradin | >2000 | 0.21 | > 9523.8 | ~~21.0~~ 15 |
| Propamocarb | > ~~2000~~ 1330 2) | 0.79 | > ~~2531.6~~ 1683.5 | ~~79.0~~ 85 |

1) Deviation [%] = 100-[(tox per fraction (a.s.*i*) - tox per fraction (mix)]/tox per fraction (a.s.*i*) × 100

2) Expressed as Propamocarb-Hydrochloride

Table ‑7: Comparison of “tox per fraction (a.s.*i*)” and “tox per fraction (mix)” for long-term/reproductive toxicity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Active substance** | **NOEL(a.s.i) [mg a.s./kg bw]** | **X (a.s.i) in the mixture** | **Tox per fraction:**  **[NOEL (a.s.i)/X (a.s.i)]** | **Contribution to overall toxicity  [%] 1)** |
| LD50 (mix) | 127.88 | 1.0 | 127.88 | - |
| Ametoctradin | 939 | 0.21 | 4471.4 | 2.9 |
| Propamocarb | 104 2) | 0.79 | 131.6 | **97.1** |

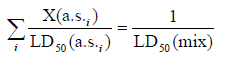
1) Contribution to overall toxicity [%] = 100-[(tox per fraction (a.s.*i*) - tox per fraction (mix)]/tox per fraction (a.s.*i*) × 100

2) Expressed as Propamocarb-Hydrochloride

For long-term/reproductive toxicity, the contribution to overall toxicity for Propamocarb is 97.1% indicating that this active substance contributes to ≥ 90 % to the long-term/reproductive toxicity of the formulationBAS 743 03 F. Therefore, the long-term/reproductive risk assessment can be performed for Propamocarb alone. Nevertheless, for completeness reasons, a long-term/reproductive risk assessment for the active substance Ametoctradin is also presented.

For acute toxicity, the contribution to overall toxicity is above 10% for both Ametoctradin and Propamocarb indicating that none of the active substances contributes to ≥ 90 % to the toxicity of formulationBAS 743 03 F. Therefore, acute risk assessment for mammals is presented for both active substances and the formulated product.

An acute endpoint from a study with the similar formulation BAS 743 02 F is available. Following Appendix B of the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA/2009/1438), the LD50 value is compared with the predicted mixture toxicity assuming dose additivity, according to the following formulation:



With:

X(a.s.i) = fraction of active substance [i] in the mixture (here: formulation)

LD50(a.s.i) = acute toxicity value for active substance [i]

LD50(mix) = measured acute toxicity value for the mixture (here: formulation)

The resulting comparison between the measured LD50 based on the LD50 values of the rat for Ametoctradin (LD50 ~~=~~ >2000 mg a.s./kg bw) and Propamocarb (LD50 = ~~2000~~ 1330 mg a.s./kg bw) with the predicted mixture toxicity assuming dose additivity is as followed:

The left-hand side of the equation (predicted mixture toxicity) is:

< ~~0.0005~~ 0.0007

And the right-hand side of the equation (measured toxicity for comparison) is:

1 / >1208.3 (product endpoint corrected for a.s. content and product density of BAS 743 02F) = <0.001

A greater value on the right side of the equation, as is the case here, indicates that the measured toxicity of a formulation is higher than predicted. In this case the use of the measured LD50 for the formulation is recommended for the first-tier assessment**.**

#### Justification for new endpoints

An additional study has been conducted on rats with the formulated product BAS 743 03 F, which has been considered for the risk assessment.

### Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

**Proposed use pattern for the risk assessments**

The proposed use pattern for the use of BAS 743 03 F is summarized in the table at the beginning of the ecotoxicology chapter (Section 9.1).

To achieve a concise risk assessment, the risk envelope approach is applied. As for Ametoctradin risk acceptability is indicated at screening step, the risk assessment is based on the worst-case crop group ~~fruiting vegetables~~ potatoes. Additionally, screening step risk assessments are presented for the worst-case scenario among the minor uses. For Propamocarb and the formulation BAS 743 03 F, risk acceptability is indicated at Tier 1/higher tier; therefore, the risk assessment is presented for all relevant crop groups. Please refer to Point 9.1.2 for further details.

The minor crops indicated in the GAP table (i.e. floriculture, avenue trees, climbing plants, conifers, ornamental shrubs, heather, forest trees and hedging plants, fruit trees and shrubs and perennial crops) are covered by the crop groups ´ornamentals´ as well as ´orchards´ and ´bush and cane fruit´ (in line with the request by the zonal Rapporteur Member State).

#### First-tier assessment (screening/generic focal species)

The results of the acute and reproductive screening risk assessments are summarised in the following table.

**Table 9.3‑8: Ametoctradin: Screening assessment of the acute and long-term/reproductive risk for mammals due to the worst-case use of BAS 743 03 F in potatoes (3 × 2.0 L product/ha)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Potatoes | | | | |
| **Active substance** | | Ametoctradin | | | | |
| **Application rate (g/ha)** | | 3 × 240 | | | | |
| **Acute toxicity (mg/kg bw)** | | > 2000 | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| ~~Bulbs and onion like crops~~, Potatoes | Small herbivorous mammal | | 118.4 | 1.79 | 50.93 | > 39.3 |
| **Reprod. toxicity (mg/kg bw/d)** | | 939 | | | | |
| **TER criterion** | | 5 | | | | |
| **Crop scenario** | **Indicator species** | | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| ~~Bulbs and onion like crops,~~ Potatoes | Small herbivorous mammal | | 48.3 | 2.21 × 0.53 | 13.56 | > 69.2 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

**Table 9.3‑9: Ametoctradin: Screening assessment of the acute and long-term/reproductive risk for mammals due to the worst-case use of BAS 743 03 F in minor uses   
(2 × 2.0 L product/ha)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Ornamentals and orchards | | | | |
| **Active substance** | | Ametoctradin | | | | |
| **Application rate (g/ha)** | | 2 × 240 | | | | |
| **Acute toxicity (mg/kg bw)** | | > 2000 | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| Ornamentals and orchards | Small herbivorous mammal | | 136.4 | 1.41 | 46.11 | > 43.4 |
| **Reprod. toxicity (mg/kg bw/d)** | | 939 | | | | |
| **TER criterion** | | 5 | | | | |
| **Crop scenario** | **Indicator species** | | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| Ornamentals and orchards | Small herbivorous mammal | | 72.3 | 1.62 × 0.53 | 14.86 | > 63.2 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The screening assessments above show~~s~~ an acceptable acute and chronic risk to mammals for the active substance Ametoctradin from the proposed uses of BAS 743 03 F. No higher tier dietary risk assessments are necessary.

**Table 9.3‑10: Propamocarb-HCL: Screening assessment of the acute and long-term/reproductive risk for mammals due to the worst-case use of BAS 743 03 F in potatoes (3 × 2.0 L product/ha)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Potatoes | | | | |
| **Active substance** | | Propamocarb | | | | |
| **Application rate (g/ha)** | | 3 × 902 | | | | |
| **Acute toxicity (mg/kg bw)** | | ~~2000~~ 1330 | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| ~~Bulbs and onion like crops,~~ Potatoes | Small herbivorous mammal | | 118.4 | 1.79 | 191.43 | ~~10.4~~ **6.9** |
| **Reprod. toxicity (mg/kg bw/d)** | | 104 | | | | |
| **TER criterion** | | 5 | | | | |
| **Crop scenario** | **Indicator species** | | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| ~~Bulbs and onion like crops,~~ Potatoes | Small herbivorous mammal | | 48.3 | 2.21 × 0.53 | 50.96 | **2.0** |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

**Table 9.3‑11: Propamocarb-HCL: Screening assessment of the acute and long-term/reproductive risk for mammals due to the worst-case use of BAS 743 03 F in minor uses (2 × 2.0 L product/ha)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Ornamentals and orchards | | | | |
| **Active substance** | | Propamocarb | | | | |
| **Application rate (g/ha)** | | 2 × 902 | | | | |
| **Acute toxicity (mg/kg bw)** | | ~~2000~~ 1330 | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| Ornamentals and orchards | Small herbivorous mammal | | 136.4 | 1.41 | 173.29 | ~~11.5~~ **7.7** |
| **Reprod. toxicity (mg/kg bw/d)** | | 104 | | | | |
| **TER criterion** | | 5 | | | | |
| **Crop scenario** | **Indicator species** | | **SVm** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| Ornamentals and orchards | Small herbivorous mammal | | 72.3 | 1.62 × 0.53 | 55.84 | **1.9** |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

The screening assessments above show~~s~~ an unacceptable acute risk to mammals for the active substance Propamocarb-HCl from the proposed uses of BAS 743 03 F. However, a potential long-term risk to mammals is indicated and therefore a first-tier long-term (reproductive) risk assessment is required.

**Table 9.3‑12: First-tier assessment of the acute and long-term risk for mammals due to the use of Propamocarb-HCl in BAS 743 03 F**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Active substance** | | | Propamocarb | | | | | | | | | |
| **Acute toxicity  (mg/kg bw/d)** | | | 1330 | | | | | | | | | |
| **TER criterion** | | | 10 | | | | | | | | | |
| **Growth stage** | | | **Generic focal species** | | **App. Rate**  **(kg a.s./ha)** | | **SVa** | | **MAFa** | | **DDDa**  **(mg/kg bw/d)** | **TERa** |
| **Orchards (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | | | | | | | |
| Application crop directed  BBCH 10-19 | | Small herbivorous mammal “vole” | | 0.902 | | 109.2 | | 1.41 | | 138.88 | | **9.58** |
| Application crop directed  BBCH 20-40 | | Small herbivorous mammal “vole” | | 81.9 | | 104.16 | | 12.77 |
| Application crop directed  BBCH ≥ 40 | | Small herbivorous mammal “vole” | | 40.9 | | 52.02 | | 25.57 |
| Application crop directed  BBCH 10-19 | | Large herbivorous mammal “lagomorph” | | 28.1 | | 35.74 | | 37.21 |
| Application crop directed  BBCH 20-40 | | Large herbivorous mammal “lagomorph” | | 21.1 | | 26.84 | | 49.55 |
| Application crop directed  BBCH ≥ 40 | | Large herbivorous mammal “lagomorph” | | 10.5 | | 13.35 | | 99.63 |
| Application crop directed  BBCH 10-19 | | Small omnivorous mammal “mouse” | | 13.8 | | 17.55 | | 75.78 |
| Application crop directed  BBCH 20-40 | | Small omnivorous mammal “mouse” | | 10.3 | | 13.10 | | 101.53 |
| Application crop directed  BBCH ≥ 40 | | Small omnivorous mammal “mouse” | | 5.2 | | 6.61 | | 201.21 |
| **Bush and cane fruit (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | | | | | | | |
| BBCH 10-19 | | Small insectivorous mammal “shrew” | | 0.902 | | 7.6 | | 1.41 | | 9.67 | | 137.54 |
| BBCH ≥ 20 | | Small insectivorous mammal “shrew” | | 5.4 | | 6.87 | | 193.60 |
| BBCH 10-19 | | Small herbivorous mammal “vole” | | 81.9 | | 104.16 | | 12.77 |
| BBCH 20-39 | | Small herbivorous mammal “vole” | | 68.2 | | 86.74 | | 15.33 |
| BBCH ≥ 40 | | Small herbivorous mammal “vole” | | 40.9 | | 52.02 | | 25.57 |
| BBCH 10-19 | | Small omnivorous mammal “mouse” | | 10.3 | | 13.10 | | 101.53 |
| BBCH 20-39 | | Small omnivorous mammal “mouse” | | 8.6 | | 10.94 | | 121.57 |
| BBCH ≥ 40 | | Small omnivorous mammal “mouse” | | 5.2 | | 6.61 | | 201.21 |
| **Active substance** | | Propamocarb | | | | | | | | | |
| **Acute toxicity  (mg/kg bw/d)** | | 1330 | | | | | | | | | |
| **TER criterion** | | 10 | | | | | | | | | |
| **Growth stage** | | | **Generic focal species** | | **App. Rate**  **(kg a.s./ha)** | | **SVa** | | **MAFa** | | **DDDm**  **(mg/kg bw/d)** | **TERa** |
| **Potatoes (3 x 0.902 kg a.s./ha, BBCH 21-89; cover 2 x 0.902 kg a.s./ha, BBCH 21-89)** | | | | | | | | | | | | |
| BBCH ≥20 | | | Small insectivorous mammal “shrew” | | 0.902 | | 5.4 | | 1.79 | | 8.72 | 152.5 |
| BBCH ≥40 | | | Small herbivorous mammal “vole” | | 40.9 | | 66.04 | 20.1 |
| BBCH 10-40 | | | Large herbivorous mammal “lagomorph” | | 14.3 | | 23.09 | 57.6 |
| BBCH ≥40 | | | Large herbivorous mammal “lagomorph” | | 35.1 | | 56.67 | 23.5 |
| BBCH 10-39 | | | Small omnivorous mammal “mouse” | | 17.2 | | 27.77 | 47.9 |
| BBCH ≥40 | | | Small omnivorous mammal “mouse” | | 5.2 | | 8.40 | 158.3 |
| **Bulbs and onion like crops (2 x 0.902 kg a.s./ha, BBCH 14–49)** | | | | | | | | | | | | |
| BBCH 10-19 | | | Small insectivorous mammal “shrew” | | 0.902 | | 7.6 | | 1.5 | | 10.28 | 129.4 |
| BBCH ≥20 | | | Small insectivorous mammal “shrew” | | 5.4 | | 7.31 | 181.9 |
| BBCH ≥40 | | | Small herbivorous mammal “vole” | | 81.9 | | 110.81 | 12.0 |
| BBCH 10-39 | | | Small omnivorous mammal “mouse” | | 17.2 | | 23.27 | 57.1 |
| BBCH ≥40 | | | Small omnivorous mammal “mouse” | | 10.3 | | 13.94 | 95.41 |
| **Active substance** | | Propamocarb | | | | | | | | | |
| **Reprod. toxicity  (mg/kg bw/d)** | | 104 | | | | | | | | | |
| **TER criterion** | | 5 | | | | | | | | | |
| **Growth stage** | | **Generic focal species** | | **App. Rate**  **(kg a.s./ha)** | | **SVm** | | **MAFm × TWA** | | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| **Potatoes (3 x 0.902 kg a.s./ha, BBCH 21-89)** | | | | | | | | | | | |
| BBCH ≥20 | | Small insectivorous mammal “shrew” | | 0.902 | | 1.9 | | 2.21 × 0.53 | | 1.99 | 52.2 |
| BBCH ≥40 | | Small herbivorous mammal “vole” | | 21.7 | | 22.76 | **4.6** |
| BBCH 10-40 | | Large herbivorous mammal “lagomorph” | | 14.3 | | 15.00 | 6.9 |
| BBCH ≥40 | | Large herbivorous mammal “lagomorph” | | 4.3 | | 4.51 | 23.1 |
| BBCH 10-39 | | Small omnivorous mammal “mouse” | | 7.8 | | 8.18 | 12.7 |
| BBCH ≥40 | | Small omnivorous mammal “mouse” | | 2.3 | | 2.41 | 43.1 |
| **Potatoes (2 x 0.902 kg a.s./ha, BBCH 21-89)** | | | | | | | | | | | |
| BBCH ≥20 | | Small insectivorous mammal “shrew” | | 0.902 | | 1.9 | | 1.71 × 0.53 | | 1.54 | 67.5 |
| BBCH ≥40 | | Small herbivorous mammal “vole” | | 21.7 | | 17.60 | 5.9 |
| BBCH 10-40 | | Large herbivorous mammal “lagomorph” | | 14.3 | | 11.60 | 9.0 |
| BBCH ≥40 | | Large herbivorous mammal “lagomorph” | | 4.3 | | 3.49 | 29.8 |
| BBCH 10-39 | | Small omnivorous mammal “mouse” | | 7.8 | | 6.33 | 16.4 |
| BBCH ≥40 | | Small omnivorous mammal “mouse” | | 2.3 | | 1.87 | 55.7 |
| **Bulbs and onion like crops (2 x 0.902 kg a.s./ha, BBCH 14–49)** | | | | | | | | | | | |
| BBCH 10-19 | | Small insectivorous mammal “shrew” | | 0.902 | | 4.2 | | 1.71 × 0.53 | | 3.41 | 30.5 |
| BBCH ≥20 | | Small insectivorous mammal “shrew” | | 1.9 | | 1.54 | 67.5 |
| BBCH ≥40 | | Small herbivorous mammal “vole” | | 43.4 | | 35.20 | **3.0** |
| BBCH 10-39 | | Small omnivorous mammal “mouse” | | 7.8 | | 6.33 | 16.4 |
| BBCH ≥40 | | Small omnivorous mammal “mouse” | | 4.7 | | 3.81 | 27.3 |
| **Fruiting vegetables (2 x 0.902 kg a.s./ha, BBCH ~~14~~ 21 - 89)** | | | | | | | | | | | |
| BBCH 71-89 | | Frugivorous mammal “rat” | | 0.902 | | 25.2 | | 1.62 × 0.53 | | 19.34 | 5.4 |
| ~~BBCH 10-19~~ | | ~~Small insectivorous mammal “shrew”~~ | | ~~4.2~~ | | ~~3.22~~ | ~~32.3~~ |
| BBCH ≥20 | | Small insectivorous mammal “shrew” | | 1.9 | | 1.46 | 71.3 |
| BBCH 10-49 | | Small herbivorous mammal “vole” | | 72.3 | | 55.50 | **1.9** |
| BBCH ≥50 | | Small herbivorous mammal “vole” | | 21.7 | | 16.66 | 6.2 |
| BBCH 10-49 | | Small omnivorous mammal “mouse” | | 7.8 | | 5.99 | 17.4 |
| BBCH ≥50 | | Small omnivorous mammal “mouse” | | 2.3 | | 1.77 | 58.9 |
| **Ornamentals (2 x 0.902 kg a.s./ha, BBCH 12–59)** | | | | | | | | | | | |
| Application to plant - exposure to underlying ground | | Small insectivorous mammal “shrew” | | 0.902 | | 1.9 | | 1.71 × 0.53 | | 1.46 | 71.3 |
| BBCH 40-49 | | Small herbivorous mammal “vole” | | 72.3 | | 55.50 | **1.9** |
| BBCH ≥50 | | Small herbivorous mammal “vole” | | 36.1 | | 27.71 | **3.8** |
| Application crop directed BBCH 10-49 | | Small omnivorous mammal “mouse” | | 7.8 | | 5.99 | 17.4 |
| Application crop directed BBCH ≥50 | | Small omnivorous mammal “mouse” | | 3.9 | | 2.99 | 34.7 |
| **~~Ornamentals (1 x 0.902 kg a.s./ha, BBCH 12–59)~~** | | | | | | | | | | | |
| ~~Application to plant - exposure to underlying ground~~ | | ~~Small insectivorous mammal “shrew”~~ | | ~~0.902~~ | | ~~1.9~~ | | ~~1.0 × 0.53~~ | | ~~0.90~~ | ~~115.2~~ |
| ~~BBCH 40-49~~ | | ~~Small herbivorous mammal “vole”~~ | | ~~72.3~~ | | ~~34.35~~ | **~~3.0~~** |
| ~~BBCH ≥50~~ | | ~~Small herbivorous mammal “vole”~~ | | ~~36.1~~ | | ~~17.15~~ | ~~6.1~~ |
| ~~Application crop directed BBCH 10-49~~ | | ~~Small omnivorous mammal “mouse”~~ | | ~~7.8~~ | | ~~3.71~~ | ~~28.1~~ |
| ~~Application crop directed BBCH ≥50~~ | | ~~Small omnivorous mammal “mouse”~~ | | ~~3.9~~ | | ~~1.85~~ | ~~56.1~~ |
| **Orchards (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | | | | | | |
| Application crop directed  BBCH 10-19 | | Small herbivorous mammal “vole” | | 0.902 | | 57.8 | | 1.62 × 0.53 | | 44.37 | **2.3** |
| Application crop directed  BBCH 20-40 | | Small herbivorous mammal “vole” | | 43.4 | | 33.31 | **3.1** |
| Application crop directed  BBCH ≥ 40 | | Small herbivorous mammal “vole” | | 21.7 | | 16.66 | 6.2 |
| Application crop directed  BBCH 10-19 | | Large herbivorous mammal “lagomorph” | | 11.5 | | 8.83 | 11.8 |
| Application crop directed  BBCH 20-40 | | Large herbivorous mammal “lagomorph” | | 8.6 | | 6.60 | 15.8 |
| Application crop directed  BBCH ≥ 40 | | Large herbivorous mammal “lagomorph” | | 4.3 | | 3.30 | 31.5 |
| Application crop directed  BBCH 10-19 | | Small omnivorous mammal “mouse” | | 6.2 | | 4.76 | 21.9 |
| Application crop directed  BBCH 20-40 | | Small omnivorous mammal “mouse” | | 4.7 | | 3.61 | 28.8 |
| Application crop directed  BBCH ≥ 40 | | Small omnivorous mammal “mouse” | | 2.3 | | 1.77 | 58.9 |
| **Bush and cane fruit (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | | | | | | |
| BBCH 10-19 | | Small insectivorous mammal “shrew” | | 0.902 | | 4.2 | | 1.62 × 0.53 | | 3.22 | 32.3 |
| BBCH ≥ 20 | | Small insectivorous mammal “shrew” | | 1.9 | | 1.46 | 71.3 |
| BBCH 10-19 | | Small herbivorous mammal “vole” | | 43.4 | | 33.31 | **3.1** |
| BBCH 20-39 | | Small herbivorous mammal “vole” | | 36.1 | | 27.71 | **3.8** |
| BBCH ≥ 40 | | Small herbivorous mammal “vole” | | 21.7 | | 16.66 | 6.2 |
| BBCH 10-19 | | Small omnivorous mammal “mouse” | | 4.7 | | 3.61 | 28.8 |
| BBCH 20-39 | | Small omnivorous mammal “mouse” | | 3.9 | | 2.99 | 34.7 |
| BBCH ≥ 40 | | Small omnivorous mammal “mouse” | | 2.3 | | 1.77 | 58.9 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

\* Covers use in bulb and onion like crops at lower application rate

The first-tier risk assessment demonstrates and acceptable long-term risk for mammals exposed to Propamocarb-HCl from the use of BAS 743 03 F except for the following scenarios:

* Potatoes (3 x 0.902 kg a.s./ha): BBCH ≥40, small herbivorous mammal “vole”
* Fruiting vegetables: BBCH 10-49, small herbivorous mammal “vole”
* Bulbs and onion like crops: BBCH ≥40, small herbivorous mammal “vole”
* Ornamentals BBCH 40-49 small herbivorous mammal “vole”
* Ornamentals BBCH ≥50 small herbivorous mammal “vole”
* Orchards BBCH 10-19 small herbivorous mammal “vole” (acute and chronic)
* Orchards BBCH 20-40 small herbivorous mammal “vole”
* Bush and cane fruit BBCH 10-19 small herbivorous mammal “vole”
* Bush and cane fruit BBCH 20-39 small herbivorous mammal “vole”

For these scenarios, further refinements are required.

**Table 9.3‑13: BAS 743 03 F: Screening assessment of the acute risk for mammals due to the worst-case use of BAS 743 03 F in potatoes (3 × 2.0 L product/ha)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Potatoes | | | | |
| **Formulation** | | BAS 743 02 F | | | | |
| **Application rate (g/ha)** | | 3 × ∑ 902 + 240 | | | | |
| **Acute toxicity (mg/kg bw)** | | > 1208.3 (based on measured LD50) | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| ~~Fruiting vegetables~~ Potato | Small herbivorous mammal | | 118.4 | 1.79 | 242.36 | **< 5.0** |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

**Table 9.3‑14: BAS 743 03 F: Screening assessment of the acute risk for mammals due to the worst-case use of BAS 743 03 F in minor uses (2 × 2.0 L product/ha)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Intended use** | | Ornamentals and orchards | | | | |
| **Formulation** | | BAS 743 02 F | | | | |
| **Application rate (g/ha)** | | 2 × ∑ 902 + 240 | | | | |
| **Acute toxicity (mg/kg bw)** | | > 1208.3 (based on measured LD50) | | | | |
| **TER criterion** | | 10 | | | | |
| **Crop scenario** | **Indicator species** | | **SV90** | **MAF90** | **DDD90**  **(mg/kg bw/d)** | **TERa** |
| Ornamentals and orchards | Small herbivorous mammal | | 136.4 | 1.41 | 219.39 | **< 5.0** |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

**Table 9.3‑15: BAS 743 03 F: First-tier assessment of the acute risk for mammals due to the use of BAS 743 03 F (1-3 × 2.0 L product/ha)**

| **Product** | BAS 743 03 F | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **Acute toxicity  (mg/kg bw/d)** | > 1208.3 (based on measured LD50) | | | | | |
| **TER criterion** | 10 | | | | | |
| **Growth stage** | **Generic focal species** | **App. Rate**  **(kg a.s./ha)** | **SV90th** | **MAF90 × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERa** |
| **Potatoes (3 × (∑ 0.240 + 0.902) 1), BBCH 21-89)** | | | | | | |
| BBCH ≥20 | Small insectivorous mammal “shrew” | 1.142 | 5.4 | 1.79 | 11.05 | > 109.3 |
| BBCH ≥40 | Small herbivorous mammal “vole” | 40.9 | 83.72 | > 14.4 |
| BBCH 10-40 | Large herbivorous mammal “lagomorph” | 35.1 | 71.85 | > 16.8 |
| BBCH ≥40 | Large herbivorous mammal “lagomorph” | 10.5 | 21.49 | > 56.2 |
| BBCH 10-39 | Small omnivorous mammal “mouse” | 17.2 | 35.21 | > 34.3 |
| BBCH ≥40 | Small omnivorous mammal “mouse” | 5.2 | 10.64 | > 113.5 |
| **Potatoes (2 × (∑ 0.240 + 0.902) 1), BBCH 21-89)** | | | | | | |
| BBCH ≥20 | Small insectivorous mammal “shrew” | 1.142 | 5.4 | 1.48 | 9.13 | > 132.3 |
| BBCH ≥40 | Small herbivorous mammal “vole” | 40.9 | 69.16 | > 17.5 |
| BBCH 10-40 | Large herbivorous mammal “lagomorph” | 35.1 | 59.35 | > 20.4 |
| BBCH ≥40 | Large herbivorous mammal “lagomorph” | 10.5 | 17.76 | > 68.1 |
| BBCH 10-39 | Small omnivorous mammal “mouse” | 17.2 | 29.09 | > 41.5 |
| BBCH ≥40 | Small omnivorous mammal “mouse” | 5.2 | 8.79 | > 137.4 |
| **Bulbs and onion like crops (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 14–49)** | | | | | | |
| BBCH 10-19 | Small insectivorous mammal “shrew” | 1.142 | 7.6 | ~~1.71~~  1.48 | 12.85 | > 94.0 |
| BBCH ≥20 | Small insectivorous mammal “shrew” | 5.4 | 9.13 | > 132.3 |
| BBCH ≥40 | Small herbivorous mammal “vole” | 81.9 | 138.49  93.53 (1 app.) | > **8.7**  12.9 |
| BBCH 10-39 | Small omnivorous mammal “mouse” | 17.2 | 29.09 | > 41.5 |
| BBCH ≥40 | Small omnivorous mammal “mouse” | 10.3 | 17.42 | > 69.4 |
| ~~BBCH ≥50~~ | ~~Small omnivorous mammal “mouse”~~ | ~~5.2~~ | ~~12.85~~ | ~~94.0~~ |
| **Fruiting vegetables (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 14 - 89)** | | | | | | |
| BBCH 71-89 | Frugivorous mammal “rat” | 1.142 | 45.2 | 1.41 | 72.70 | > 16.6 |
| BBCH 10-19 | Small insectivorous mammal “shrew” | 7.6 | 12.22 | > 98.8 |
| BBCH ≥20 | Small insectivorous mammal “shrew” | 5.4 | 8.69 | > 139.1 |
| BBCH 10-49 | Small herbivorous mammal “vole” | 136.4 | 219.39 | > **5.5** |
| BBCH ≥50 | Small herbivorous mammal “vole” | 40.9 | 65.79 | > 18.4 |
| BBCH 10-49 | Small omnivorous mammal “mouse” | 17.2 | 27.67 | > 43.7 |
| BBCH ≥50 | Small omnivorous mammal “mouse” | 5.2 | 8.36 | > 144.5 |
| **Ornamentals (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 12–59)** | | | | | | |
| Application to plant - exposure to underlying ground | Small insectivorous mammal “shrew” | 1.142 | 5.4 | 1.41 | 8.69 | > 139.1 |
| BBCH 40-49 | Small herbivorous mammal “vole” | 136.4 | 219.39 | > **5.5** |
| BBCH ≥50 | Small herbivorous mammal “vole” | 68.2 | 109.70 | > 11.0 |
| Application crop directed BBCH 10-49 | Small omnivorous mammal “mouse” | 17.2 | 27.67 | > 43.7 |
| Application crop directed BBCH ≥50 | Small omnivorous mammal “mouse” | 8.6 | 13.83 | > 87.4 |
| **~~Ornamentals (1 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 12–59)~~** | | | | | | |
| ~~Application to plant - exposure to underlying ground~~ | ~~Small insectivorous mammal “shrew”~~ | ~~1.142~~ | ~~5.4~~ | ~~1.0~~ | ~~6.16~~ | ~~177.9~~ |
| ~~BBCH 40-49~~ | ~~Small herbivorous mammal “vole”~~ | ~~136.4~~ | ~~155.72~~ | **~~7.0~~** |
| ~~BBCH ≥50~~ | ~~Small herbivorous mammal “vole”~~ | ~~68.2~~ | ~~77.86~~ | ~~14.1~~ |
| ~~Application crop directed BBCH 10-49~~ | ~~Small omnivorous mammal “mouse”~~ | ~~17.2~~ | ~~19.64~~ | ~~55.9~~ |
| ~~Application crop directed BBCH ≥50~~ | ~~Small omnivorous mammal “mouse”~~ | ~~8.6~~ | ~~9.82~~ | ~~111.7~~ |
| **Orchards (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| Application crop directed  BBCH 10-19 | Small herbivorous mammal “vole” | 1.142 | 109.2 | 1.41 | 175.64 | > **6.9** |
| Application crop directed  BBCH 20-40 | Small herbivorous mammal “vole” | 81.9 | 131.73 | > **9.2** |
| Application crop directed  BBCH ≥ 40 | Small herbivorous mammal “vole” | 40.9 | 65.79 | > 18.4 |
| Application crop directed  BBCH 10-19 | Large herbivorous mammal “lagomorph” | 28.1 | 45.20 | > 26.7 |
| Application crop directed  BBCH 20-40 | Large herbivorous mammal “lagomorph” | 21.1 | 33.94 | < 35.6 |
| Application crop directed  BBCH ≥ 40 | Large herbivorous mammal “lagomorph” | 10.5 | 16.98 | > 71.5 |
| Application crop directed  BBCH 10-19 | Small omnivorous mammal “mouse” | 13.8 | 22.20 | > 54.4 |
| Application crop directed  BBCH 20-40 | Small omnivorous mammal “mouse” | 10.3 | 16.57 | > 72.9 |
| Application crop directed  BBCH ≥ 40 | Small omnivorous mammal “mouse” | 5.2 | 8.36 | > 144.5 |
| **Bush and cane fruit (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 12 - 59)** | | | | | | |
| BBCH 10-19 | Small insectivorous mammal “shrew” | 1.142 | 7.6 | 1.41 | 12.22 | > 98.8 |
| BBCH ≥ 20 | Small insectivorous mammal “shrew” | 5.4 | 8.69 | > 139.1 |
| BBCH 10-19 | Small herbivorous mammal “vole” | 81.9 | 131.73 | > **9.2** |
| BBCH 20-39 | Small herbivorous mammal “vole” | 68.2 | 109.70 | > 11.0 |
| BBCH ≥ 40 | Small herbivorous mammal “vole” | 40.9 | 65.79 | > 18.4 |
| BBCH 10-19 | Small omnivorous mammal “mouse” | 10.3 | 16.57 | > 72.9 |
| BBCH 20-39 | Small omnivorous mammal “mouse” | 8.6 | 13.83 | > 87.4 |
| BBCH ≥ 40 | Small omnivorous mammal “mouse” | 5.2 | 8.36 | > 144.5 |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

1) Sum of the application rates of the individual active substances*, i.e*. Ametoctradin (0.240 kg a.s./ha) and Propamocarb (*i.e*. 0.902 kg Propamocarb/ha)

2) Measured LD50 (mix) corrected for density of the product and content of total active substance

The first-tier risk assessment demonstrates and acceptable acute risk for mammals exposed to BAS 743 03 F for all intended uses except for the following scenarios:

* Bulbs and onion like crops: BBCH ≥40, small herbivorous mammal “vole”
* Fruiting vegetables: BBCH 10-49, small herbivorous mammal “vole”
* Ornamentals BBCH 40-49 small herbivorous mammal “vole”
* Orchards BBCH 10-19 small herbivorous mammal “vole”
* Orchards BBCH 20-40 small herbivorous mammal “vole”
* Bush and cane fruit BBCH 10-19 small herbivorous mammal “vole”

However, it needs to be considered that the risk assessment for the formulation BAS 743 03 F is conducted based on the measured toxicity endpoint of LD50 > 2000 mg product/kg bw/d corrected for the total amount of active substance and the product density resulting in a LD50 > 1208.3 mg total a.s./kg bw/d. Since this endpoint is higher than the highest dose tested and no effects (*i.e.,* no mortality, no effects on weight gained and no macroscopic pathological findings in any animal sacrificed at the end of the observation period) were observed, it can be concluded that the formulation BAS 74303 F is non-toxic by oral route in rats. In addition, both active substances, Ametoctradin and Propamocarb-HCl, show low toxicity to rats ~~with both LD~~~~50~~ ~~= 2000 mg/kg bw/d.~~ Therefore, an acceptable acute risk to mammals exposed to BAS 743 03 F is demonstrated without the need for a higher tier risk assessment. ~~However, as a conservative approach, an acute higher tier risk assessment is presented below based on refined deposition factors.~~

#### Higher-tier risk assessment

According to the above first-tier risk assessment, a long-term higher-tier risk assessments is required for Propamocarb-HCl for small herbivorous mammals (vole) in potatoes at 3 x 0.902 kg a.s./ha, BBCH ≥40, BBCH 40 – 49, fruiting vegetables, BBCH 10-49, ornamentals BBCH 40-49 and BBCH >50, bulbs and onion like crops at 2 x 0.902 kg a.s./ha, BBCH ≥40, orchards at 2 x 0.902 kg a.s./ha, BBCH 10-19 and BBCH 20-40 as well as in bush and cane fruit at 2 x 0.902 kg a.s./ha, BBCH 10-19.

**Small herbivorous mammal “vole”**

***Focal species***

Although small herbivorous mammals, represented by the “vole”, are listed as a potentially relevant generic focal species in EFSA/2009/1438, it is widely acknowledged that voles are not relevant (*i.e*. no actual ´focal species´) for field crops for the following reasons:

• High fecundity and population recuperation

• Forage for their primary source of food outside crop fields

* Necessity of population control measures since the vole is considered a crop pest when high population levels are reached

Accordingly, the risk to small mammals is considered to be covered by small omnivorous mammals (“wood mouse”), for which an acceptable risk was demonstrated with a large margin of safety in the first-tier risk assessment.

In addition, the common vole as a realistic representative of the small herbivorous feeding guild, while occurring in agricultural field is confined to habitats with significant crop coverage. The optimum or prime habitat includes undisturbed grassland with a short vegetation cover consisting of a wide variety of dicotyledonous and monocotyledonous plant species (Meunier et al. 1994). However, in the cropping of potatoes, onions, ornamentals and fruiting vegetables, weed control is an important component of production. This will significantly limit the presence of grasses in the field. Thus, it can be assumed that the relevant source populations of voles predominantly inhabit off-crop habitats such as meadows or grassy areas which represented relevant foraging areas and habitats for these small mammals. Two focal species studies are available in the RAR for Propamocarb (2017). One study was conducted to study the attractiveness of arable fields cropped with leafy vegetables (RAR Propamocarb, Vol. 3 – CP B9, June 2017). The study was performed in spring and early summer in major cabbage growing areas in Germany and The Netherlands and it was confirmed that vegetable fields do not constitute a primary habitat for voles since voles were trapped early and more frequently in the surroundings than in cropped fields. The second field study performed on cereals (RAR Propamocarb, Vol. 3 – CP B9, June 2017) confirmed that these annual crops do not constitute primary habitats but may be secondary habitats for the Common vole. This and the low recapture rates determined for cereal fields indicate that voles immigrating into arable fields are not able to establish stable populations. Indeed, the regular harvesting of annual crops necessarily limits this phenomenon, voles looking for food and stable habitat.

However, as a conservative approach a refined risk assessment for small herbivorous mammals (represented by the vole) is presented below considering refined exposure and a refined portion of diet (PD).

**Refinement: Deposition factor**

According to Appendix A of the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) it is assumed that small herbivorous mammals (vole) are feeding on 100% grass in treated fields. It is not assumed that voles will feed on the treated crops (potatoes, fruiting vegetables, ornamentals and onions), but on any weeds present below the treated plants. Since the food item is at ground level, it is possible to refine the default deposition factor of 1. Within Appendix E of the EFSA Guidance on Bird and Mammal Risk Assessment on ‘Impact of crop interception on residues on plant food items’, in referring to deposition estimates for Tier I, states that ‘The deposition factors provided for the different crops and growth stages are likely to reflect conservative estimates. In the context of a higher-tier assessment, the more detailed values of FOCUS Ground Water Guidance Assessment (Version 2.2; May 2014) may therefore also be used’. Therefore, this risk assessment will be refined as applicable using FOCUS Groundwater guidance interception values (EFSA 2014). At BBCH ≥40, the leaf cover in the treated crop is estimated to intercept approximately 70% of residues for leafy vegetables (which can also be used as a surrogate for ornamentals), 40% of residues for onions (in line with Tier 1 assumptions), 80% of residues for fruiting vegetables and 85% of residues for potatoes. Thus, the deposition factor (DF) for use in the higher tier assessment calculation is 0.30, 0.60, 0.2 and 0.15 for ornamentals, onions, fruiting vegetables and potatoes, respectively at BBCH ≥ 40 (see Part B, Section 8). In fruiting vegetables, the minimum interception to be assumed for BBCH 10-19 is 50% resulting in a deposition factor of 0.5. In orchards and bush and cane fruit as surrogate crops for the intended minor uses, the ´crop´ interception according to FOCUS Ground Water Guidance for the intended crop growth stages is 60% resulting in a deposition factor of 0.4 (as compared to factors of 0.8 for BBCH 10-19 in orchards, 0.6 at BBCH 20-40 in orchards and at BBCH 10-19 in bush and cane fruit, respectively).

|  |
| --- |
| **Review Comments:**  According to “Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology” (May 2021), point 3.2.15, the interception values following EFSA Guidance Document to obtain DegT50 values (EFSA Journal 2014;12(5):3662), can be use in the Tier 2 risk assessment. It should be noted that this rule applies only to the later stages of crop growth.  In Appendix E of EFSA B&M guidance the following recommendation is given:  *“It was concluded that estimation of residues on undergrowth vegetation using FOCUS interception factors would become increasingly uncertain with decreasing soil cover of the crop and increasing height of weeds in relation to the crop. Thus reliable predictions are only deemed possible where the largest part of the soil surface is actually covered by the crop from a bird’s eye view and undergrowth vegetation is clearly smaller than the crop plants. Weeds or grasses overgrowing the crop at those stages are deemed unlikely to occur in intensive agriculture, but would anyway not form a part of the diet of small to medium herbivores.”*  In Table 1 Appendix E for solanaceous fruit and cucurbits BBCH ≥ 51 growth stages are recognized satisfactorily high soil coverage by crop plants.  In Table 1.5 of EFSA Guidance Document to obtain DegT50 values for BBCH 40-89 growth stages of tomatoes the interception is 80%. It is the highest percentage of ground cover for fruiting vegetables.  There is no justification for the different approach to IF for BBCH stages ≥ 40 and ≥51. Therefore, in zRMS opinion, for BBCH growth stages of tomatoes ≥ 40, the DF of 0.2 can be used in the risk assessment. |

**Refinement: DT50 of Propamocarb-HCl**

The first-tier risk assessment is based on a default foliar DT50 of 10 days. However, residue field data are available which demonstrate that Propamocarb-HCl degrades rapidly in foliage. In order to determine DT50 values, residues trials with sufficiently short sampling intervals are required (e.g. Day 0, 3, 7 and 14). Such data are available, submitted to support the EU review (RAR Propamocarb, Vol. 3 – CP B9, June 2017). These are considered typical of the vegetation of the intended uses of BAS 743 03 F and thus the data derived from this study will be applicable to other crop types such as fruiting vegetables (tomatoes, aubergine and cucurbits) and ornamentals.

Given the extensive care dedicated to the ground cultivation in potato, onion and ornamental fields, it is unlikely that tall grasses represented by mature cereals would typically be found. Therefore, the DT50 value for residue dissipation of Propamocarb-HCl determined on young and small cereals is considered relevant for the exposure scenario of voles feeding on short grass in potato, onion and fruiting vegetable fields (if available). Based on residue data in/on wheat at early growth stages (BBCH 25-33), a DT50 for residue dissipation of Propamocarb-HCl of 3.32 days (RAR Propamocarb, Vol. 3 – CP B9, June 2017) was determined.

With the refined DT50 value of 3.32 days instead of the default value of 10 days, new MAF×twa values for Propamocarb-HCl for the intended use in potatoes, fruiting vegetables, ornamentals and onions, respectively were calculated with the moving-time window approach for 3 applications and 5-day interval according to the formula detailed in Appendix H of the EFSA Guidance Document (2009). This MAF×twa value can be used for refined TER calculation (see Table 9.3 - 14).

|  |
| --- |
| **Review Comments:**  ~~The Applicant's proposal for the use of DT~~~~50~~ ~~value of 3.32 days for monocots was accepted.~~  According to RAR 12.2022 the study of residue dissipation of Propamocarb-HCl in wheat plants, in which a DT50 value in wheat was determined in four trials, was stated by experts at Pesticide Peer Review Meeting PREV 03 (March 2019) as being not sufficient to support the refinement of the long-term risk assessment for mammals based on residue decline in wheat used as surrogate for grass/weeds since the number of trials is too low to cover all EU zones. Thus, the TER calculation with refined DT50 were deleted. |

| **~~Active substance~~** | ~~Propamocarb~~ | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **~~Reprod. toxicity  (mg/kg bw/d)~~** | ~~104~~ | | | | | |
| **~~TER criterion~~** | ~~5~~ | | | | | |
| **~~Growth stage~~** | **~~Generic focal species~~** | **~~App. Rate~~**  **~~(kg a.s./ha)~~** | **~~SV~~~~m~~** | **~~MAF~~~~m~~ ~~× TWA~~** | **~~DDD~~~~m~~**  **~~(mg/kg bw/d)~~** | **~~TER~~~~lt~~** |
| **~~Potatoes (3 x 0.902 kg a.s./ha, BBCH 21-89)~~** | | | | | | |
| ~~BBCH ≥40~~ | ~~Small herbivorous mammal “vole”~~ | ~~0.902~~ | ~~21.7~~ | ~~0.65~~ | ~~12.72~~ | ~~8.18~~ |
| **~~Bulbs and onion like crops (2 x 0.902 kg a.s./ha, BBCH 14–49)~~** | | | | | | |
| ~~BBCH ≥40~~ | ~~Small herbivorous mammal “vole”~~ | ~~0.902~~ | ~~43.4~~ | ~~0.45~~ | ~~17.62~~ | ~~5.90~~ |
| **~~Fruiting vegetables (2 x 0.902 kg a.s./ha, BBCH 21 - 89)~~** | | | | | | |
| ~~BBCH 10-49~~ | ~~Small herbivorous mammal “vole”~~ | ~~0.902~~ | ~~72.3~~ | ~~0.44~~ | ~~28.69~~ | **~~3.62~~** |
| **~~Ornamentals (2 x 0.902 kg a.s./ha, BBCH 12–59)~~** | | | | | | |
| ~~BBCH 40-49~~ | ~~Small herbivorous mammal “vole”~~ | ~~0.902~~ | ~~72.3~~ | ~~0.44~~ | ~~28.62~~ | **~~3.62~~** |
| ~~BBCH ≥50~~ | ~~Small herbivorous mammal “vole”~~ | ~~36.1~~ | ~~14.33~~ | ~~7.26~~ |
| **~~Orchards (2 x 0.902 kg a.s./ha, BBCH 12 - 59)~~** | | | | | | |
| ~~Application crop directed~~  ~~BBCH 10-19~~ | ~~Small herbivorous mammal “vole”~~ | ~~0.902~~ | ~~57.8~~ | ~~0.44~~ | ~~22.94~~ | **~~4.53~~** |
| ~~Application crop directed~~  ~~BBCH 20-40~~ | ~~Small herbivorous mammal “vole”~~ | ~~43.4~~ | ~~17.22~~ | ~~6.04~~ |
| **~~Bush and cane fruit (2 x 0.902 kg a.s./ha, BBCH 12 - 59)~~** | | | | | | |
| ~~BBCH 10-19~~ | ~~Small herbivorous mammal “vole”~~ | ~~0.902~~ | ~~43.4~~ | ~~0.44~~ | ~~17.22~~ | ~~6.04~~ |
| ~~BBCH 20-39~~ | ~~Small herbivorous mammal “vole”~~ | ~~36.1~~ | ~~14.33~~ | ~~7.26~~ |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

\* Covers use in bulb and onion like crops at lower application rate

**Refinement: PD**

1. **Composition of food items in the diet (PD)**

The diet of the vole in the Tier 1 assessment according to the EFSA Bird and Mammal Guidance Document (2009) is 100 % grasses (*i.e.* monocots). However, this is an unrealistic worst-case assumption. Studies have demonstrated that common voles (*Microtus arvalis*) prefer to consume dicotyledons (broad leaf weeds) rather than monocotyledonous grasses. Leutert (1983)[[3]](#footnote-3) investigated the food piles of voles and taking the average of the preferences in the two meadow types investigated (fertilized and unfertilized meadows) resulted in a vole diet of 44.5% mono- and 55.5% dicotyledonous plants.

This is similar to data obtained from grassland in Germany (Rinke, 1990 and 1991) [[4]](#footnote-4)[[5]](#footnote-5), where the diet of 363 individuals caught by snap-trapping was examined through analysis of stomach contents. The results showed that dicotyledons, such as *Taraxacum officinale* and *Trifolium pratense*, were preferred and were eaten at a higher frequency than would be expected from their relative occurrence in the grassland habitat in question. Overall, it was reported that dicotyledons comprised a mean volume percentage of 63.5% of stomach contents of common vole. Therefore, the risk assessment can be still conservatively refined by considering a common vole consuming a diet comprising 60% dicotyledons (non-grass herbs) and 40% grasses.

Based on a diet of 60% dicotyledons (non-grass herbs) and 40% grasses the food intake rate for a common vole (body weight 25 g) was calculated.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Review Comments:**  ~~For the purposes of this assessment, the ecological data on PD and FIR/bw for common vole are not taken to consideration, due to the lack of a harmonised approach to Rinke, 1990.~~  ~~Therefore, new TER calculations without changed of this factor were performed.~~  At the harmonization meeting of the central zone in December 2023, it was agreed that for crops other than orchards, vines, hops, grassland and cereals, the PD of 0.50 and 0.50 for monocots and dicots, respectively, can be accepted in refined risk assessment for voles. The new FIR/bw is 1.46.  **Calculations of new shortcut value based on data from EFSA B&M guidance (i.e. bw and RUDs)**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Indicator/generic focal species** | **Typ of food** | **RUDmean** | **PD** | **SVmix** | | Small herbivorous mammal "vole” | Monocotyledons | 54.2 | 0.50 | 27.1 | | Dicotyledonos | 28.7 | 0.50 | 14.35 | |  | | | 1.0 | 41.45 | | | | | | | | | | |
| **Active substance** | | Propamocarb | | | | | | | | |
| **Reprod. toxicity  (mg/kg bw/d)** | | 104 | | | | | | | | |
| **TER criterion** | | 5 | | | | | | | | |
| **App. Rate**  **(kg a.s./ha)** | | 0.902 | | | | | | | | |
| **Growth stage** | | **Generic focal species** | **FIR/bw** | | **DF** | | **RUDmix** | **MAFm × TWA** | **DDDm**  **(mg/kg bw/d)** | **TERlt** |
| **Potatoes (3 x 0.902 kg a.s./ha, BBCH 21-89)** | | | | | | | | | | |
| BBCH ≥40 | | Small herbivorous mammal “vole” | 1.46 | 0.3 | | | 41.45 | 2.21 x 0.53 | 19.18 | 5.4 |
| **Bulbs and onion like crops (2 x 0.902 kg a.s./ha, BBCH 14–49)** | | | | | | | | | | |
| BBCH ≥40 | | Small herbivorous mammal “vole” | 1.46 | | 0.6 | | 41.45 | 1.71 × 0.53 | 29.68 | **3.5** |
| **Bulbs and onion like crops (1 x 0.902 kg a.s./ha, BBCH 14–49)** | | | | | | | | | | |
| BBCH ≥40 | | Small herbivorous mammal “vole” | 1.46 | | 0.6 | | 41.45 | 0.53 | 17.36 | 6.0 |
| **Fruiting vegetables (2 x 0.902 kg a.s./ha, BBCH 21 - 89)** | | | | | | | | | | |
| BBCH 10-49 | | Small herbivorous mammal “vole” | 1.46 | | 1 | | 41.45 | 1.62 × 0.53 | 46.87 | **2.2** |
| **Ornamentals (2 x 0.902 kg a.s./ha, BBCH 12–59)** | | | | | | | | | | |
| BBCH 40-49 | | Small herbivorous mammal “vole” | 1.46 | | 1 | | 41.45 | 1.71 × 0.53 | 49.47 | **2.1** |
| BBCH ≥50 | | Small herbivorous mammal “vole” | 1.46 | | 0.3# | | 41.45 | 1.71 × 0.53 | 14.84 | 7.0 |
| **Orchards (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | | | | | |
| Application crop directed  BBCH 10-19 | | Small herbivorous mammal “vole” | 1.46 | | | 0.8 | 41.45 | 1.62 × 0.53 | 37.49 | **2.8** |
| Application crop directed  BBCH 20-40 | | Small herbivorous mammal “vole” | 1.46 | | | 0.6 | 41.45 | 1.62 × 0.53 | 28.12 | **3.7** |
| **Bush and cane fruit (2 x 0.902 kg a.s./ha, BBCH 12 - 59)** | | | | | | | | | | |
| BBCH 10-19 | | Small herbivorous mammal “vole” | 1.46 | | | 0.6 | 41.45 | 1.62 × 0.53 | 28.12 | **3.7** |
| BBCH 20-39 | | Small herbivorous mammal “vole” | 1.46 | | | 0.5 | 41.45 | 1.62 × 0.53 | 23.43 | **4.4** |

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

#DF for cabbage

**~~Table 9.3‑16:  FIR based on refined PD values~~**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Species~~** | **~~Food type~~** | **~~Energetic content of food~~**  **~~(kJ/g wet weight)~~** | **~~Assimilation efficiency (%)~~** | **~~Energetic content of food weighed by assimilation efficiency~~**  **~~(kJ/g wet weight)~~** | **~~Proportion of food item in mixed diet (%)~~** | **~~Energy uptake per food item in mixed diet~~**  **~~(kJ/g wet weight)~~** | **~~DEE (kJ)~~** | **~~Daily consumption per diet item~~**  **~~(g wet weight/day)~~** | **~~Relative FIR~~**  **~~(g wet weight/bw/d)~~** |
| ~~Common vole~~  ~~(bw = 25 g)~~ | ~~Grasses and cereal shoots~~ | ~~4.15~~ | ~~47~~ | ~~1.95~~ | ~~40~~ | ~~0.78~~ |  | ~~14.91~~ | ~~0.596~~ |
| ~~Non-grass herbs~~ | ~~2.12~~ | ~~76~~ | ~~1.61~~ | ~~60~~ | ~~0.97~~ |  | ~~22.36~~ | ~~0.984~~ |
| **~~Total~~** |  |  |  | **~~100~~** | **~~1.75~~** | **~~65.09~~** | **~~37.27~~** | **~~1.490~~** |

~~1) App. A: EFSA Journal 2009; 7(12):1438, mammal tier 1 tables; 2) calculated according to app. G: EFSA Journal 2009; 7(12):1438; 3) See App. G: EFSA Journal 2009; 7(12):1438, Table 3; 4) See App. G EFSA Journal 2009; 7(12):1438, Table 4~~

**~~Table 9.3‑17: Higher-tier assessment of the long-term/reproductive risk for mammals due to the use of BAS 743 03 F – refined parameters (\*) are further described and justified in the text~~**

| **~~Active substance~~** | | ~~Propamocarb-HCl~~ | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Reproductive toxicity (mg/kg bw/d)~~** | | ~~104~~ | | | | | | | | | |
| **~~TER criterion~~** | | ~~5~~ | | | | | | | | | |
| **~~GAP crop~~** | **~~Application rate~~**  **~~(g a.s./ha)~~** | **~~Crop~~**  **~~scenario~~**  **~~Growth stage~~**  **~~(BBCH)~~** | **~~Generic Focal species~~** | **~~FIR/ bw~~** | **~~Diet item~~** | **~~RUD~~~~m~~** | **~~PD~~** | **~~DF~~** | **~~MAF~~~~m~~ ~~x TWA~~** | **~~DDD~~~~m~~**  **~~(mg/kg~~**  **~~bw/d)~~** | **~~TER~~~~LT~~** |
| ~~Potatoes~~ | ~~3 x 902~~ | ~~≥40~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.49~~  ~~\*~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~0.40~~  ~~\*~~ | ~~0.15\*~~ | ~~0.65\*~~ | ~~5.10~~ | ~~20.39~~ |
| ~~Non-grass herbs~~ | ~~28.7~~ | ~~0.60~~  ~~\*~~ |
| ~~Onions~~ | ~~2 x 902~~ | ~~≥ 40~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.49\*~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~0.40\*~~ | ~~0.60\*~~ | ~~0.45\*~~ | ~~14.12~~ | ~~7.36~~ |
| ~~Non-grass herbs~~ | ~~28.7~~ | ~~0.60\*~~ |
| ~~Fruiting vegetables~~ | ~~2 x 902~~ | ~~10-49~~  ~~≥40~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.49~~  ~~1.33~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~0.40~~  ~~1~~ | ~~0.5 0.2\*~~ | ~~0.44\*~~ | ~~11.51~~  ~~6.34~~ | ~~9.04~~  ~~16.4~~ |
| ~~Non-grass herbs~~ | ~~28.7~~ | ~~0.60~~  ~~\*~~ |
| ~~Ornamentals~~ | ~~2 x 902~~ | ~~40-49 / ≥ 50~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.49~~  ~~1.33~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~0.40~~  ~~1~~ | ~~0.3\*~~ | ~~0.44\*~~ | ~~6.90~~  ~~8.58~~ | ~~15.06~~  ~~12.12~~ |
| ~~Non-grass herbs~~ | ~~28.7~~ | ~~0.60~~  ~~\*~~ |
| ~~Ornamentals~~ | ~~1 x 902~~ | ~~40-49~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.49\*~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~0.40\*~~ | ~~0.3\*~~ | ~~0.23\*~~ | ~~3.61~~ | ~~28.82~~ |
| ~~Orchards~~ | ~~2 x 902~~ | ~~10-19~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.33~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~1~~ | ~~0.8\*\*~~ | ~~0.44\*~~ | ~~22.89~~ | **~~4.54~~** |
| ~~Orchards~~ | ~~2 x 902~~ | ~~10 20-40~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.49~~  ~~1.33~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~0.40~~  ~~1~~ | ~~0.4\*~~  ~~0.6\*\*~~ | ~~0.44\*~~ | ~~9.20~~  ~~17.17~~ | ~~11.30~~  ~~6.06~~ |
| ~~Non-grass herbs~~ | ~~28.7~~ | ~~0.60~~ |
| ~~Bush and cane fruit~~ | ~~2 x 902~~ | ~~10-39~~ | ~~Small herbivorous mammal "vole”~~ | ~~1.49~~  ~~\*~~ | ~~Grasses and cereal shoots~~ | ~~54.2~~ | ~~0.40\*~~ | ~~0.4\*~~ | ~~0.44\*~~ | ~~9.20~~ | ~~11.30~~ |
| ~~Non-grass herbs~~ | ~~28.7~~ | ~~0.60~~  ~~\*~~ |

~~FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.~~

~~\* Refined parameters~~

~~\*\*DF form EFSA B&M 2009~~

An acceptable risk can be concluded by the refined higher tier assessment for the chronic risks to mammals, except for multiple applications in onions BBCH ≥ 40, ornamentals BBCH 40-49, orchards BBCH 10-40, bush and cane fruit BBCH 10-39 and fruiting vegetables BBCH ≤ 49.

**~~Formulation BAS 743 03 F:~~**

**~~Table 9.3‑18: Higher-tier assessment of the acute risk for mammals due to the use of BAS 743 03 F – refined parameters (\*) are further described and justified in the text~~**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Active substance/product~~** | ~~BAS 743 03 F~~ | | | | | | |
| **~~Application rate [kg/ha]~~** | ~~2 × ∑ 0. 240 + 0.902~~ | | | | | | |
| **~~Acute toxicity [mg/kg bw]~~** | ~~> 1208.3 (based on measured LD~~~~50~~~~)~~ | | | | | | |
| **~~TER criterion~~** | ~~10~~ | | | | | | |
| **~~Bulbs and onion like crops (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 14 - 89)~~** | | | | | | | |
| **~~Crop scenario~~**  **~~Growth stage~~** | **~~Generic focal species~~** | **~~FIR/bw~~** | **~~RUD~~**  **~~[mg/kg]~~** | **~~MAF~~~~90~~** | **~~DF~~** | **~~DDD~~~~90~~**  **~~[mg/kg bw/d]~~** | **~~TER~~~~A~~** |
| ~~Onions BBCH> 40~~ | ~~Small herbivorous mammal “vole”~~ | ~~1.33~~ | ~~102.3~~ | ~~1.48~~ | ~~0.6\*~~ | ~~138.36~~ | **~~8.7~~** |
| **~~Fruiting vegetables (2 × (∑ 0.240 + 0.902), BBCH 14 21-89)~~** | | | | | | | |
| **~~Crop scenario~~**  **~~Growth stage~~** | **~~Generic focal species~~** | **~~FIR/bw~~** | **~~RUD~~**  **~~[mg/kg]~~** | **~~MAF~~~~90~~** | **~~DF~~** | **~~DDD~~~~90~~**  **~~[mg/kg bw/d]~~** | **~~TER~~~~A~~** |
| ~~Fruiting vegetables BBCH 10-49~~ | ~~Small herbivorous mammal “vole”~~ | ~~1.33~~ | ~~102.3~~ | ~~1.41~~ | ~~0.5\*~~ | ~~109.8~~ | ~~11.0~~ |
| **~~Ornamentals (2 × (∑ 0.240 + 0.902), BBCH 12-59)~~** | | | | | | | |
| **~~Crop scenario~~**  **~~Growth stage~~** | **~~Generic focal species~~** | **~~FIR/bw~~** | **~~RUD~~**  **~~[mg/kg]~~** | **~~MAF~~~~90~~** | **~~DF~~** | **~~DDD~~~~90~~**  **~~[mg/kg bw/d]~~** | **~~TER~~~~A~~** |
| ~~Ornamentals BBCH 40-49~~ | ~~Small herbivorous mammal “vole”~~ | ~~1.33~~ | ~~102.3~~ | ~~1.41~~ | ~~0.3\*~~ | ~~34.93~~ | ~~18.3~~ |
| **~~Ornamentals (1 × (∑ 0.240 + 0.902), BBCH 12-59)~~** | | | | | | | |
| **~~Crop scenario~~**  **~~Growth stage~~** | **~~Generic focal species~~** | **~~FIR/bw~~** | **~~RUD~~**  **~~[mg/kg]~~** | **~~MAF~~~~90~~** | **~~DF~~** | **~~DDD~~~~90~~**  **~~[mg/kg bw/d]~~** | **~~TER~~~~A~~** |
| ~~Ornamentals BBCH 40-49~~ | ~~Small herbivorous mammal “vole”~~ | ~~1.33~~ | ~~102.3~~ | ~~1.0~~ | ~~0.3\*~~ | ~~24.77~~ | ~~48.78~~ |
| **~~Orchards (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 12 - 59)~~** | | | | | | | |
| **~~Crop scenario~~**  **~~Growth stage~~** | **~~Generic focal species~~** | **~~FIR/bw~~** | **~~RUD~~**  **~~[mg/kg]~~** | **~~MAF~~~~90~~** | **~~DF~~** | **~~DDD~~~~90~~**  **~~[mg/kg bw/d]~~** | **~~TER~~~~A~~** |
| ~~Orchards BBCH 10-40~~ | ~~Small herbivorous mammal “vole”~~ | ~~1.33~~ | ~~102.3~~ | ~~1.41~~ | ~~0.4\*~~ | ~~87.88~~ | ~~13.8~~ |
| **~~Bush and cane fruit (2 x (∑ 0.240 + 0.902) kg a.s./ha, BBCH 12 - 59)~~** | | | | | | | |
| **~~Crop scenario~~**  **~~Growth stage~~** | **~~Generic focal species~~** | **~~FIR/bw~~** | **~~RUD~~**  **~~[mg/kg]~~** | **~~MAF~~~~90~~** | **~~DF~~** | **~~DDD~~~~90~~**  **~~[mg/kg bw/d]~~** | **~~TER~~~~A~~** |
| ~~Bush and cane fruit BBCH 10-39~~ | ~~Small herbivorous mammal “vole”~~ | ~~1.33~~ | ~~102.3~~ | ~~1.41~~ | ~~0.4\*~~ | ~~87.88~~ | ~~13.8~~ |

~~FIR/bw: Food intake rate per body weight; RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.~~

~~\* Refined parameters~~

~~For the formulation BAS 74 03 F, an acceptable risk can be concluded by the refined higher tier assessment for the acute risks to mammals. Only the TER for bulb and onion like crops remains below the trigger of 10. However, with reference to the argumentation provided above concerning the ecology of voles and specifically the preference of more natural habitats, overall, the risk for small herbivorous mammals is considered to be acceptable.~~

#### Drinking water exposure

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)OC of 3779 L/kg and of 535.56 L/kg for Ametoctradin and Propamocarb, respectively both active substances belong to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group potatoes also covers the risk for mammals from all other intended uses in fruiting vegetables, and bulbs and onion like crops. The ratio calculations for effective application rate to relevant endpoints are detailed in Table 9.3-15 and Table 9.2-16.

Table ‑19: Assessment of the risk for mammals due to exposure to Ametoctradin via contaminated drinking water in puddles

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Ametoctradin** | **Reference** |
| Koc (geometric mean) [L/kg] | 3779 | Chapter 8.5.1 and 8.5.2 |
| Number of applications | 3 | See above |
| Interval [days] | 5 | See above |
| MAFm 1) | 2.21 | -- |
| Max use rate [g/ha] | 240 | See above |
| AReff [g/ha] 2) | 530 | -- |
| LD50 [mg/kg b.w.] | 2000 | See above |
| Ratio (acute) 3) | 0.265 | -- |
| NO(A)EL [mg/kg b.w./d] | 939 | See above |
| Ratio (repro) 3) | 0.564 | -- |
| Trigger | 3000 | -- |
| Drinking water assessment  required [Yes/No] | No | -- |

1) MAFm = (1-e-nki) / (1-e-ki) with k = ln(2)/DT50 (rate constant), n = number of applications and i = application interval [d]

2) AReff = Application rate (g/ha) x MAFmean

3) Ratio of AReff and relevant toxicity endpoint

Table ‑20: Assessment of the risk for mammals due to exposure to Propamocarb-HCl via contaminated drinking water in puddles

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Propamocarb-HCl** | **Reference** |
| Koc (geometric mean) [L/kg] | 535.56 | Chapter 8.5.1 and 8.5.2 |
| Number of applications | 3 | See above |
| Interval [days] | 5 | See above |
| MAFm 1) | 2.21 | -- |
| Max use rate [g/ha] | 902 | See above |
| AReff [g/ha] 2) | 1991 | -- |
| LD50 [mg/kg b.w.] | 2000 | See above |
| Ratio (acute) 3) | 0.995 | -- |
| NO(A)EL [mg/kg b.w./d] | 104 | See above |
| Ratio (repro) 3) | 19.14 | -- |
| Trigger | 3000 | -- |
| Drinking water assessment  required [Yes/No] | No | -- |

1) MAFm = (1-e-nki) / (1-e-ki) with k = ln(2)/DT50 (rate constant), n = number of applications and i = application interval [d]

2) AReff = Application rate (g/ha) x MAFmean

3) Ratio of AReff and relevant toxicity endpoint

In conclusion, the risk to mammals via drinking water from the intended use of BAS 743 03 F according to the proposed use pattern is acceptable.

#### Effects of secondary poisoning

The log Pow of the active substance Ametoctradin is 4.4 (XXXX DocID 2005/1014072), which triggers an assessment of the potential risk from secondary poisoning.

The log Pow of Propamocarb-HCl amounts to -1.2 (pH = 7, EFSA Scientific Report (2006)) and thus does not exceed the trigger value of 3.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil is based on experimental data.

As shown in the following table, the TERLT for ametoctradin exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning.

Table ‑21: Assessment of the risk for earthworm-eating mammals due to exposure to Ametoctradin via bioaccumulation in earthworms (secondary poisoning) for the worst-case intended use in onion like crops

| **Parameter** | Ametoctradin | **Reference** |
| --- | --- | --- |
| PECsoil (twa, 21 days)[mg/kg soil] 1) | ~~0.033~~ 0.145 | Chapter 8.7 (Table 8.7-6) |
| Kow | 25119 | XXXX DocID 2005/1014072 |
| Koc (arithmetic mean) | 3779 | EFSA Journal 2012, 10(11): 2921;  XXXX DocID 2008/1017000 and 2008/1046556 |
| foc (default) | 0.02 | EFSA/2009/1438 |
| BCFearthworm | 4.013 | -- |
| PECworm [mg/kg] | ~~0.132~~ 0.582 | PECworm = PECsoil x BCFearthworm |
| Daily dose [mg/kg b.w./d] | ~~0.170~~ 0.745 | Daily dose = PECworm x 1.28 |
| NO(A)EL [mg/kg b.w./d] | 939.0 | See above |
| TERlt | ~~5539.1~~ 1260 | -- |

1) Highest PECsoil (21-d, twa) as worst-case, selected from 1 application in onion as a risk envelope.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

As shown in the following table, the TERLT for ametoctradin exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating mammals via secondary poisoning.

Table ‑22: Assessment of the risk for fish-eating birds due to exposure to Ametoctradin via bioaccumulation in fish (secondary poisoning) for the worst-case intended use in potato

| **Parameter** | **Ametoctradin** | **Reference** |
| --- | --- | --- |
| PECsw, (initial) [mg/L] 1) | 0.1645 | Chapter 8.9 (Step 1) |
| BCF fish (max. worst case) | 219 | EFSA Journal 2012, 10(11): 2921 |
| PECfish [mg/kg] | 36.026 | PECfish = PECwater x TWA x BCF |
| Daily dose [mg/kg b.w./d] | 5.116 | Daily dose = PECfish x 0.142 |
| NO(A)EL [mg/kg b.w./d] | 939.0 | See above |
| TERlt | 183.6 | -- |

1) Highest PECsw (initial) as worst-case, selected from twofold application scenario in potatoes (FOCUS Step 1). For details please refer to chapter 8.9.

#### Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU reviews of Ametoctradin (EFSA Scientific Report (2012) 10(11): 2921) and Propamocarb-HCl (EFSA Scientific Report (2006)) from toxicokinetics studies.

Since the bioaccumulation potential of Ametoctradin and Propamocarb is low, no further assessment on biomagnification along the food chain is required.

### Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

### Overall conclusions

It can be concluded that the risk to mammals from the application of BAS 743 03 F according to good agricultural practice is acceptable.

|  |
| --- |
| **Review Comments:**  The acute and chronic risks of BAS 743 03 F to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items. An acute oral toxicity study with BAS 743 02 F in rats was taken to consideration in the evaluation.  All TER values exceed the relevant triggers in the screening step risk assessment for ametoctradin (acute and chronic).  All acute TER values exceed the relevant triggers in the Tier 1 risk assessment for propamocarb-HCl except for uses in surrogate crop scenario: orchards BBCH 10-19.  Based on the higher tier chronic risk assessment for propamocarb-HCl, where the deposition factor and ~~DT~~~~50~~ ~~in plants~~ PD for voles were modified, the TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects except for uses in fruiting vegetables at BBCH 11-39, multiple applications in onions BBCH ≥ 40, ornamentals BBCH 40-49, and for surrogate crops scenarios: orchards BBCH 10-40 and bush and cane fruit BBCH 10-39.  Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is low. |

## Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

According to regulations (EU) 283/2013 and 284/2013, the risk to amphibians and reptiles shall be addressed. However, in the EU there are no guidance documents by EFSA on how to conduct risk assessments for amphibians and reptiles. Nevertheless, there are some specific recommendations in an EU regulatory document (EFSA aquatic guidance), which are taking here in consideration to address the requirements under point 9.4.

The aquatic guidance document (EFSA, 2013) states: “*Even if the revised data requirements (Commission Regulation (EU) 283/2013) do not request toxicity tests for amphibian species, amphibians should be included in the aquatic and terrestrial RA of PPPs. Assessment of the risk to amphibians should be based on any existing relevant information. Available relevant data, including data from the open literature, for the substance under consideration should be presented and taken into account in the RA…*”. Therefore, the availability of studies on the toxicity of the active substances should be considered.

For both active substances contained in the formulation BAS 743 03 F, i.e. Ametoctradin and Propamocarb-HCl, there are no studies available, neither in the literature nor unpublished reports by the notifier, on their toxicity to amphibians or reptiles. Therefore, due to the lack of a standard risk assessment and of data on the toxicity of the active substances to amphibian and reptiles, a regulatory risk assessment for these organisms is not applicable at this time.

## Effects on aquatic organisms (KCP 10.2)

### Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with the active substances Ametoctradin and Propamocarb. In addition, aquatic studies were performed with the Ametoctradin metabolites M650F01, M650F03 and M650F04. Full details of these studies are provided in the respective EU DAR and related documents. M650F02 was not tested because it is an intermediate metabolite with similar chemical structure as M650F01 and/or M650F03.

A new life-cycle study with the Mysid shrimp (*Americamysis bahia*) with Ametoctradin has been submitted. This is listed in Appendix 1 and summarised in Appendix 2.

Effects on aquatic organisms of BAS 743 03 F were not evaluated as part of the EU assessment of the active substances Ametoctradin and Propamocarb. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Toxicity testing of BAS 743 03 F has been performed with the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) on one species from each three groups of aquatic organisms, *i.e.* fish (*Oncorhynchus mykiss*), aquatic invertebrates (*Daphnia magna*) and algae (*Pseudokirchneriella subcapitata*). Since differences in co-formulants and/or their concentration between both formulations are considered minimal, it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission. In addition, toxicity testing of BAS 743 03 F has been performed on aquatic invertebrates (*Daphnia magna*, BAS Doc ID 2022/2033730) indicating no increased toxicity of BAS 743 03 F compared to BAS 743 02 F based on the content of active substance.

Chronic studies on fish and invertebrates for the formulation were not conducted, since there is no indication that the formulation is more acutely toxic than the two active substances by a factor of 10. Thus, it is possible to extrapolate from chronic toxicity data obtained in the corresponding studies on the active substances.

The selection of studies and endpoints for the risk assessment for both Ametoctradin and Propamocarb-HCl is in line with the results of the EU review process, except for the consideration of the new Mysid study.

**Table 9.5‑1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Ametoctradin and relevant metabolites**

| **Species** | **Substance** | **Exposure**  **System** | **Results** | **Reference / XXXX DocID** |
| --- | --- | --- | --- | --- |
| *Oncorhynchus mykiss* | Ametoctradin | 96 h, f | LC50 > 0.0646 mg a.s./L  mean measured | EFSA Conclusion 2012 2007/1004041 |
| *Oncorhynchus mykiss* | Ametoctradin **\***  (tested as BAS 650 00 F) | 96 h, s | **LC50 > 19.0 mg a.s./L**  mean measured | EFSA Conclusion 2012 2007/1057733 |
| *Cyprinus carpio* | Ametoctradin | 96 h, f | LC50 > 0.110 mg a.s./L  mean measured | EFSA Conclusion 2012 2007/1039553 |
| *Lepomis macrochirus* | Ametoctradin | 96 h, f | LC50 > 0.129 mg a.s./L  mean measured | EFSA Conclusion 2012 2006/1031686 |
| *Pimephales promelas* | Ametoctradin | 33 d, f | **NOEC = 0.048 mg a.s./L**  mean measured | EFSA Conclusion 2012 2006/1024627 |
| *Daphnia magna* | Ametoctradin | 48 h, s | EC50 > 0.590 mg/L  mean measured | EFSA Conclusion 2012 2006/1037557 |
| *Daphnia magna* | Ametoctradin **\***  (tested as BAS 650 00 F) | 48 h, s | **EC50 > 19.4 mg/L**  mean measured | EFSA Conclusion 2012 2007/1018762 |
| *Daphnia magna* | Ametoctradin | 21 d, ss | NOEC = 0.044 mg/L  mean measured | EFSA Conclusion 2012 2007/1057496 |
| *Americamysis bahia* | Ametoctradin | 28 d, f | **NOEC = 0.018 mg/L**  mean measured | New study  2013/7000443 |
| *Chironomus riparius* | Ametoctradin **\***  (tested as BAS 650 00 F) | 28 d, spiked sediment | **NOEC = 221.56 mg a.s./kg sed. (dw)**  initial measured | EFSA Conclusion 2012 2007/1057455 |
| *Pseudokirchneriella subcapitata* | Ametoctradin | 72 h, s | **ErC50/EyC50 > 0.118 mg a.s./L**  mean measured | EFSA Conclusion 2012 2008/1034458 |
| *Pseudokirchneriella subcapitata* | Ametoctradin **\***  (tested as BAS 650 00 F) | 72 h, s | **ErC50/EyC50 > 18.4 mg a.s./L**  nominal | EFSA Conclusion 2012 2007/1017586 |
| *Daphnia magna* | Metabolite: M650F01 | 48 h, s | **EC50 > 100 mg a.s./L**  nominal | EFSA Conclusion 2012 2008/1034472 |
| *Oncorhynchus mykiss* | Metabolite: M650F03 | 96 h, s | **LC50 > 82.60 mg a.s./L**  nominal | EFSA Conclusion 2012 2007/1035788 |
| *Daphnia magna* | Metabolite: M650F03 | 48 h, s | **EC50 > 82.60 mg a.s./L**  nominal | EFSA Conclusion 2012 2007/1035785 |
| *Daphnia magna* | Metabolite: M650F03 | 21 d, ss | **NOEC = 41.75 mg a.s./L**  nominal | EFSA Conclusion 2012 2008/1043909 |
| *Pseudokirchneriella subcapitata* | Metabolite: M650F03 | 72 h, s | **ErC50/EyC50 > 82.60 mg a.s./L**  nominal | EFSA Conclusion 2012 2007/1035786 |
| *Oncorhynchus mykiss* | Metabolite: M650F04 | 96 h, s | **LC50 > 100 mg a.s./L**  nominal | EFSA Conclusion 2012 2007/1035789 |
| *Daphnia magna* | Metabolite: M650F04 | 48 h, s | **EC50 > 100 mg a.s./L**  nominal | EFSA Conclusion 2012 2007/1035784 |
| *Pseudokirchneriella subcapitata* | Metabolite: M650F04 | 72 h, s | **ErC50/EyC50 > 100 mg a.s./L**  nominal | EFSA Conclusion 2012 2007/1035787 |

s: static; ss: semi-static; f: flow-through

**Bold** figures: Endpoint used in aquatic risk assessment.

\* Study was conducted with the solo-formulation BAS 650 00 F; the endpoints obtained for the solo-formulation BAS 650 00 F have been converted to active substance ametoctradin (considering the analysed contents of the a.s., (i.e. 192 g a.s./L for the fish, *Daphnia* and alga study; 204.4 g a.s./L for the *Chironomus* study) and the formulation density of 1.041 g/cm3) and are used in the following risk assessment of the a.s.; for details please refer to the updated DAR of ametoctradin (Vol. 3, Annex B.9, December 2010).

**Table 9.5‑2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Propamocarb-HCl**

| **Species** | **Substance** | **Exposure**  **System** | **Results** | **Reference / XXXX DocID** |
| --- | --- | --- | --- | --- |
| *Oncorhynchus mykiss* | Propamocarb-HCl | 96 h, f | LC50 > 99 mg a.s./L  mean measured | EFSA Scientific Report (2006) |
| *Lepomis macrochirus* | Propamocarb-HCl | 96 h, f | **LC50 > 92 mg a.s./L**  **mean measured** | EFSA Scientific Report (2006) |
| *Lepomis macrochirus* | Propamocarb-HCl | 32 d, f | **NOEC >6.3 mg a.s./L**  mean measured | EFSA Scientific Report (2006) |
| *Daphnia magna* | Propamocarb-HCl | 48 h, s | **EC50 > 100 mg/L**  **mean measured** | EFSA Scientific Report (2006) |
| *Daphnia magna* | Propamocarb-HCl | 21 d, ss | **NOEC = 12.3 mg/L**  mean measured | EFSA Scientific Report (2006) |
| *Pseudokirchneriella subcapitata* | Propamocarb-HCl | 72 h, s | **ErC50 > 85 mg a.s./L**  mean measured | EFSA Scientific Report (2006) |
| *Lemna gibba* | Propamocarb-HCl | 14 d, s | **EC50 > 18 mg a.s./L**  nominal | EFSA Scientific Report (2006) |

s: static; ss: semi-static; f: flow-through

**Bold** figures: Endpoint used in aquatic risk assessment.

**Table 9.5‑3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – BAS 743 03 F**

| **Species** | **Substance** | **Exposure**  **System** | **Results** | **Reference / XXXX DocID** |
| --- | --- | --- | --- | --- |
| *Oncorhynchus mykiss* | BAS 743 02 F\* | 96 h, s | **LC50 > 100 mg/L**  **nominal**  > 60.42 mg Σ a.s./L nom 1)  > 68.58 mg Σ a.s./L mm 2) | New study  2022/2033714 |
| *Daphnia magna* | BAS 743 02 F\* | 48 h, s | EC50 > 100 mg/L  nominal  > 60.42 mg Σ a.s./L nom 1)  > 59.53 mg Σ a.s./L mm 3) | New study  2022/2033712 |
| *Daphnia magna* | BAS 743 03 F | 48 h, ss | **EC50 > 100 mg/L**  **nominal**  > 53.3 Σ mg a.s./L nom 2)  > 55.31 Σ mg a.s./L mm 3) | New study  2022/2033730 |
| *Pseudokirchneriella subcapitata* | BAS 743 02 F\* | 72 h, s | **ErC50 > 100 mg/L**  **nominal**  > 60.42 mg Σ a.s./L nom 1)  > 60.54 mg Σ a.s./L mm 2) | New study  2022/2033713 |

**Bold** figures: Endpoint used in aquatic risk assessment.

s: static, ss: semi-static

\* Studies were conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl

1) Calculated based on the total content of active substances in the product (652.5 g/L) and the product density of 1.080 g/cm3

2) Calculated based on the total content of active substances in the product (571 g/L) and the product density of 1.071 g/cm3

3) Calculated following EFSA (2019); endpoint based on the “sum of active substances” geomean concentration levels

**Mixture toxicity**

In line with EFSA (2013), in addition to measured toxicity data, mixture toxicity is assessed on the basis of the Concentration Addition (CA) model. A surrogate endpoint for CA is calculated using the following equation:



where:

n = number of mixture components

pi = the ith component as a relative fraction of the mixture composition (∑ pi must be 1)

ECxi = concentration of component i provoking x% effect

In order to determine if the a.s. may act more (*i.e*. synergistically) or less (*i.e.* antagonistically) than expected by concentration addition (CA), a comparison of the calculated ECxmix-CA for the mixture composition of a.s. in the formulation versus measured ECxPPP endpoints has been conducted.

In addition, to determine if the active substances may act more (*i.e.* synergistically) or less (*i.e.* antagonistically) than expected by CA, the measured formulation toxicity (ECxPPP) is compared against the calculated mixture toxicity ECxmix-CA for exactly the mixture composition of the active substances in the formulation (ECxPPP) by means of the Model Deviation Ratio (MDR):



The deviations between calculated and measured mixture toxicity, in terms of the model deviation ratio (MDR) are presented in the following table.

The observed and calculated mixture toxicity is considered in agreement if the MDR is between 0.2 and 5. More-than additive (i.e. synergistic) mixture toxicity is indicated if the MDR is > 5. Less-than additive (*i.e*. antagonistic) mixture toxicity is indicated if the MDR is below 0.2.

**Table 9.5‑4: Estimation of mixture L(E)C50, contribution to overall toxicity and MDR for fish, invertebrates and algae assuming concentration additivity (proportion of active substances in the formulation)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Organism** | **Time scale** | **Toxicity endpoint** | **Test substance** | **Toxicity endpoint**  **[µg a.s./L]** | **Toxicity per fraction for CA/Surrogate endpoint**  **[µg a.s./L]** | **Product endpoint corrected for active substance content and density**  **[µg a.s./L]1)** | **Contribution to overall toxicity [%]** | **MDR** |
| Fish | acute | LC50 | Ametoctradin | 19000 | 90426.7 | 60416.7**1)** | 56.3 | 0.843 |
| Propamocarb-HCl | 92000 | 116472.6 | 43.7 |
| BAS 743 02 F | 100000 | **50905.1** | n.a. |
| chronic | NOEC | Ametoctradin | 48 | 228.6 | -- | **97.21** | -- |
| Propamocarb-HCl | 6300 | 7974.7 | 2.786 |
| BAS 743 02 F | n.a. | **222.2** | n.a. |
| Aquatic invertebrates | acute | EC50 | Ametoctradin | 19400 | 92330.4 | 53314.7**2)** | 57.83 | 1.0 |
| Propamocarb-HCl | 100000 | 126600.7 | 42.17 |
| BAS 743 03 F | 100000 | **53391.7** | n.a. |
| chronic | NOEC | Ametoctradin | 18 | 85.7 | -- | **99.45** | -- |
| Propamocarb-HCl | 12300 | 15569.6 | 0.548 |
| BAS 743 02 F | n.a. | **85.2** | n.a. |
| Algae | chronic | EC50 | Ametoctradin | 18400 | 87571.1 | 60416.7**1)** | 55.12 | 0.80 |
| Propamocarb-HCl | 85000 | 107610.6 | 44.88 |
| BAS 743 02 F | 100000 | **48281.1** | n.a. |

MDR: Model deviation ratio; n.a. not applicable/available

1. Based on an active substance content of 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl and a product density of 1.080 g/cm3
2. 1) Based on an active substance content of 120 g/L Ametoctradin and 451 g/L Propamocarb-HCl and a product density of 1.071 g/cm3

The resulting MDRs presented above are all between 0.2 and 5, indicating that the observed and calculated mixture toxicities are considered to be in agreement. Thus, the measured mixture toxicity endpoints have been used in the following risk assessment for drift entry for the formulated product.

The active substance Ametoctradin in case of the ratio of active substances as in the formulated product contributes by more than 90% to the chronic toxicity of the formulation BAS 743 03 F to fish and aquatic invertebrates; thus the risk assessment for these trophic level is based on the single-substance toxicity data of Ametoctradin. No “driver” of acute mixture toxicity is identified for fish, aquatic invertebrates and algae; thus, any potential risk due to the acute toxicity of BAS 743 03 F is addressed in a mixture risk assessment following the Risk Quotient Approach (RQ) using the following equation.



The RQmix approach is fully compatible with the principal assessment scheme as in accordance with EFSA guidance, *i.e*., the assessments based on ratios of PECSW and RAC, whereas EFSA guidance proposes the calculation of ETRs. Furthermore, the RQ approach is also presented in the guidance as an alternative option for the ETR calculation.

The mixture toxicity assessment is presented in Table 9.5-11 for the intended worst-case use at 2 x 1.75 L/ha in potatoes. The assessment is based on worst-case maximum PECSW values at FOCUS Steps 1 and 2.

#### Justification for new endpoints

Agreed endpoints for Ametoctradin and its metabolites were taken from the EFSA Conclusion (2012). In addition, a new chronic study on the Mysid shrimp has been submitted, which provides the lowest endpoint (NOEC = 0.018 mg a.s./L) for the chronic risk assessment.

New endpoints with the formulated product (BAS 743 02 F and BAS 743 03 F) have been presented, as this is a different product to that supported for the EU evaluation.

### Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of 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”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

In accordance with the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters” (EFSA Aquatic GD), risk assessment for algae was performed considering the more relevant endpoint “growth rate” (ErC50).

**Ametoctradin (BAS 650 F)**

The relevant global maximum FOCUS PECsw and PECsed for risk assessments covering the proposed use patterns and the resulting PEC/RAC ratios are presented in the tables below.

For full details of the assumptions used in the exposure calculations please see Part B, Section 8. FOCUS PECSW and PECSED for ametoctradin and its relevant metabolites are presented in Section 8, Chapter 8.9.

The acute studies conducted with ametoctradin resulted in endpoints which are greater than the water solubility of the compound under test conditions. Solubility of ametoctradin in distilled water is 0.15 mg a.s./L at 20°C and pH 7 (XXXX DocID 2005/1014832). It has to be considered that water solubility in test media may differ from the values derived in distilled water at a certain pH and temperature. Accordingly, the lowest acute endpoint was observed in the study with rainbow trout due to low water temperature and thus lower solubility of the test item. In summary, the acute testing showed no or only slight effects at the saturation concentration of ametoctradin. Therefore, it was decided to include studies with the solo formulated product BAS 650 00 F (nominal content of ametoctradin: 200 g a.s./L), indicating an overall low to moderate toxicity. In the following risk assessment, PEC/RAC ratio calculations were based on the endpoints derived with the formulated product (based on the content of the active substance).

**Propamocarb**

The relevant global maximum FOCUS PECsw for risk assessments covering the proposed use patterns and the resulting PEC/RAC ratios are presented in the tables below (see

Table .

For full details of the assumptions used in the exposure calculations, please see Part B, Section 8. FOCUS PECSW for Propamocarb are presented in Section 8, Chapter 8.9. Only worst-case PECSW for each use/crop group are considered for Propamocarb (covering early and late applications during spring, summer or winter).

**BAS 743 03 F**

The relevant global maximum FOCUS PECmix for risk assessments covering the proposed use patterns and the resulting PEC/ECxmix ratios are presented in the tables below (see Table 9.5‑.5)

As indicated in Point 9.5.1, the chronic toxicity of the formulation BAS 743 03 F to aquatic organismsis clearly driven by the toxicity of Ametoctradin. Thus, the chronic risk assessment is based on single-substance toxicity and exposure data on this active substance. However, none of the active substances, *i.e*. Ametoctradin or Propamocarb, drives the acute toxicity of the formulation BAS 743 03 F. Therefore, the acute risk assessment is presented based on the RQmix approach (EFSA Aquatic Guidance Document, 2013)

**Active substance Ametoctradin:**

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PECSW, PECSED) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for each FOCUS scenario and each organism group for the active substance Ametoctradin for the intended worst-case uses in potatoes, tomatoes, ornamentals and salad crops/herbs at 1-3 x 2.0 L product/ha.

Table 9.5‑5 Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Ametoctradin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the worst-case use of BAS 743 03 F in potatoes (3 × 2.0 L/ha)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | | **Fish acute** | **Fish chronic** | **Inverteb. acute** | | **Inverteb. chronic** | | **Algae** | |  | **Sed. dwell. chronic** |
| **Test species** | | ***O. mykiss \**** | ***P. promelas*** | ***D. magna*** | ***D. magna \**** | ***D. magna*** | ***A. bahia*** | ***P. subcapitata*** | ***P. subcapitata \**** | ***C. riparius \**** |
| **Endpoint [µg/L]** | | LC50 | NOEC | EC50 | EC50 | NOEC | NOEC | ErC50 | ErC50 | NOEC |
| > 19000 | 48 | > 590 | > 19400 | 44 | 18 | > 118 | > 18400 | 221560 |
| **AF** | | 100 | 10 | 100 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC [µg/L]** | | > 190 | 4.8 | > 5.9 | > 194 | 4.4 | 1.8 | > 11.8 | > 1840 | 22156 |
| **FOCUS Scenario** | **PECsw gl-max 1) [µg/L]** | **PEC/ RAC ratio** | | | | | | | | **PECsed. gl-max [µg/kg]** | **PEC/ RAC ratio** |
| **Step 1** | 16.452 | 0.09 | **3.43** | **2.79** | 0.08 | **3.74** | **9.14** | **1.39** | 0.01 | 493.162 | 0.02 |
| **Step 2** | | | | | | | | | | | |
| N-Europe | 2.207 | 0.01 | 0.46 | 0.37 | 0.01 | 0.50 | **1.23** | 0.19 | 0.00 | -- | -- |
| S-Europe | 2.207 | 0.01 | 0.46 | 0.37 | 0.01 | 0.50 | **1.23** | 0.19 | 0.00 | -- | -- |
| **Step 3** | | | | | | | | | | | |
| D3/ditch | 1.245 | -- | 0.26 | -- | -- | -- | 0.69 | -- | -- | -- | -- |
| D4/pond | 0.049 | -- | 0.01 | -- | -- | -- | 0.03 | -- | -- | -- | -- |
| D4/stream | 0.97 | -- | 0.20 | -- | -- | -- | 0.54 | -- | -- | -- | -- |
| D6/ditch | 1.235 | -- | 0.26 | -- | -- | -- | 0.69 | -- | -- | -- | -- |
| D6/ditch | 1.237 | -- | 0.26 | -- | -- | -- | 0.69 | -- | -- | -- | -- |
| R1/pond | 0.049 | -- | 0.01 | -- | -- | -- | 0.03 | -- | -- | -- | -- |
| R1/stream | 0.861 | -- | 0.18 | -- | -- | -- | 0.48 | -- | -- | -- | -- |
| R2/stream | 1.155 | -- | 0.24 | -- | -- | -- | 0.64 | -- | -- | -- | -- |
| R3/stream | 1.215 | -- | 0.25 | -- | -- | -- | 0.68 | -- | -- | -- | -- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

\* Study was conducted with the solo-formulation BAS 650 00 F; endpoint is given as mean measured concentration of the active substance.

1) Worst-case PEC value resulting from calculations for single or multiple application.

**Table 9.5‑6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Ametoctradin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of BAS 743 03 F in tomatoes and aubergine (2 × 2.0 L/ha)**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | | **Fish acute** | **Fish chronic** | **Inverteb. acute** | | **Inverteb. chronic** | | **Algae** | |  | **Sed. dwell. chronic** |
| **Test species** | | ***O. mykiss \**** | ***P. promelas*** | ***D. magna*** | ***D. magna \**** | ***D. magna*** | ***A. bahia*** | ***P. subcapitata*** | ***P. subcapitata \**** | ***C. riparius \**** |
| **Endpoint [µg/L]** | | LC50 | NOEC | EC50 | EC50 | NOEC | NOEC | ErC50 | ErC50 | NOEC |
| > 19000 | 48 | > 590 | > 19400 | 44 | 18 | > 118 | > 18400 | 221560 |
| **AF** | | 100 | 10 | 100 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC [µg/L]** | | > 190 | 4.8 | > 5.9 | > 194 | 4.4 | 1.8 | > 11.8 | > 1840 | 22156 |
| **FOCUS Scenario** | **PECsw gl-max 1) [µg/L]** | **PEC/ RAC ratio** | | | | | | | | **PECsed. gl-max [µg/kg]** | **PEC/ RAC ratio** |
| **Step 1** | 16.452 | 0.09 | **3.43** | **2.79** | 0.08 | **3.74** | **9.14** | **1.39** | 0.01 | 493.162 | 0.02 |
| **Step 2** | | | | | | | | | | | |
| N-Europe | 2.207 | -- | 0.46 | 0.37 | -- | 0.50 | **1.23** | 0.19 | -- | -- | -- |
| S-Europe | 2.207 | -- | 0.46 | 0.37 | -- | 0.50 | **1.23** | 0.19 | **--** | -- | -- |
| **Step 3** | | | | | | | | | | | |
| D6/ditch | 1.486 | -- | 0.31 | -- | -- | -- | 0.83 | -- | -- | -- | -- |
| R2/stream | 1.332 | -- | 0.28 | -- | -- | -- | 0.74 | -- | -- | -- | -- |
| R3/stream | 1.401 | -- | 0.29 | -- | -- | -- | 0.78 | -- | -- | -- | -- |
| R4/stream | 0.993 | -- | 0.21 | -- | -- | -- | 0.55 | -- | -- | -- | -- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

\* Study was conducted with the solo-formulation BAS 650 00 F; endpoint is given as mean measured concentration of the active substance.

1) Worst-case PEC value resulting from calculations for single or multiple application in tomatoes and aubergine.

Table 9.5‑7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Ametoctradin for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of BAS 743 03 F in onions (2 × 2.0 L/ha)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | | **Fish acute** | **Fish chronic** | **Inverteb. acute** | | **Inverteb. chronic** | | **Algae** | |  | **Sed. dwell. chronic** |
| **Test species** | | ***O. mykiss \**** | ***P. promelas*** | ***D. magna*** | ***D. magna \**** | ***D. magna*** | ***A. bahia*** | ***P. subcapitata*** | ***P. subcapitata \**** | ***C. riparius \**** |
| **Endpoint [µg/L]** | | LC50 | NOEC | EC50 | EC50 | NOEC | NOEC | ErC50 | ErC50 | NOEC |
| > 19000 | 48 | > 590 | > 19400 | 44 | 18 | > 118 | > 18400 | 221560 |
| **AF** | | 100 | 10 | 100 | 100 | 10 | 10 | 10 | 10 | 10 |
| **RAC [µg/L]** | | > 190 | 4.8 | > 5.9 | > 194 | 4.4 | 1.8 | > 11.8 | > 1840 | 22156 |
| **FOCUS Scenario** | **PECsw gl-max 1) [µg/L]** | **PEC/ RAC ratio** | | | | | | | | **PECsed. gl-max [µg/kg]** | **PEC/ RAC ratio** |
| **Step 1** | 16.452 | 0.09 | **3.43** | **2.79** | 0.08 | **3.74** | **9.14** | **1.39** | 0.01 | 493.162 | 0.02 |
| **Step 2** | | | | | | | | | | | |
| N-Europe | 2.207 | -- | 0.46 | 0.37 | -- | 0.50 | **1.23** | 0.19 | -- | -- | -- |
| S-Europe | 2.207 | -- | 0.46 | 0.37 | -- | 0.50 | **1.23** | 0.19 | **--** | -- | -- |
| **Step 3** | | | | | | | | | | | |
| D3/ditch | 1.504 | -- | 0.31 | -- | -- | -- | 0.31 | -- | -- | -- | -- |
| D4/pond | 0.051 | -- | 0.01 | -- | -- | -- | 0.01 | -- | -- | -- | -- |
| D4/stream | 1.147 | -- | 0.24 | -- | -- | -- | 0.24 | -- | -- | -- | -- |
| D6/ditch | 1.516 | -- | 0.32 | -- | -- | -- | 0.32 | -- | -- | -- | -- |
| D6/ditch | 1.501 | -- | 0.31 | -- | -- | -- | 0.31 | -- | -- | -- | -- |
| R1/pond | 0.051 | -- | 0.01 | -- | -- | -- | 0.01 | -- | -- | -- | -- |
| R1/stream | 0.992 | -- | 0.21 | -- | -- | -- | 0.21 | -- | -- | -- | -- |
| R2/stream | 1.311 | -- | 0.27 | -- | -- | -- | 0.27 | -- | -- | -- | -- |
| R3/stream | 1.399 | -- | 0.29 | -- | -- | -- | 0.29 | -- | -- | -- | -- |
| R4/stream | 0.984 | -- | 0.21 | -- | -- | -- | 0.21 | -- | -- | -- | -- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

\* Study was conducted with the solo-formulation BAS 650 00 F; endpoint is given as mean measured concentration of the active substance.

1) Worst-case PEC value resulting from calculations for single or multiple application in onion.

For the intended use in potatoes, tomatoes, salad crops, herbs and onions at 1 - 2 × 1.75 L/ha, calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for invertebrates as characterised by a NOEC for *A. bahia* in connection with an assessment factor of 10) based on the FOCUS Step 3 PECSW values. Therefore, no further assessment is necessary.

**Active substance Propamocarb-HCl:**

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PECSW, PECSED) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for each FOCUS scenario and each organism group for the active substance Propamocarb-HCl for the intended worst-case uses in potatoes, cucurbits, tomatoes and salad crops/herbs at 2 x 1.75 L product/ha.

Table 9.5‑8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Propamocarb-HCl for each organism group based on FOCUS Steps 1 and 2 calculations for the worst-case use of BAS 743 03 F in potatoes (3 × 2.0 L/ha)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | | **Fish acute** | **Fish prolonged** | **Inverteb. acute** | **Inverteb. prolonged** | **Algae** | **Aquatic macrophytes** |
| **Test species** | | ***L. macrochirus*** | ***L. macrochirus*** | ***D. magna*** | ***A. bahia*** | ***P. subcapitata*** | *Lemna gibba* |
| **Endpoint [µg/L]** | | LC50 | NOEC | EC50 | NOEC | ErC50 | ErC50  > 18000 |
| > 92000 | 6300 | > 100000 | 12300 | > 85000 |
| **AF** | | 100 | 10 | 100 | 10 | 10 | 10 |
| **RAC [µg/L]** | | > 920.0 | 630.0 | > 1000 | 1230 | > 8500 | > 1800 |
| **FOCUS Scenario** | **PEC gl-max 1) [µg/L]** | **PEC/ RAC ratio** | | | | | |
| **Step 1** | 692.309 | 0.75 | **1.10** | 0.69 | 0.56 | 0.08 | 0.38 |
| **Step 2** | | | | | | | |
| N-Europe | 119.108 | 0.13 | 0.19 | 0.12 | 0.10 | 0.01 | 0.07 |
| S-Europe | 97.360 | 0.11 | 0.15 | 0.10 | 0.08 | 0.01 | 0.05 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**.

For the intended worst-case uses in potatoes (covering the other uses), calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for chronic fish as characterised by a NOEC of 6.3 mg a.s./L for *Lepomis macrochirus* in connection with an assessment factor of 10) based on the FOCUS Step 1 PECSW values. Therefore, no further assessment is necessary.

**Metabolites of Ametoctradin**

Ecotoxicological studies were performed with the metabolites M650F01, M650F03 and M650F04. M650F02 was not tested because it is an intermediate metabolite with similar chemical structure as M650F01 and/or M650F03. Hence, M650F02 is not included in the following aquatic risk assessment calculations, though PEC values are available in chapter Section 8, Chapter 8.9.

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PECSW) and regulatory acceptable concentrations (RAC) for aquatic organisms are given.

Table 9.5‑9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of ametoctradin for each organism group based on FOCUS Step 1 calculations for the worst-case use of BAS 743 03 F in potatoes, fruiting vegetables and onions (1 - 3 x 2.0 L/ha)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test species** | | ***O. mykiss***  **(acute)** | ***D. magna***  **(acute)** | ***D. magna***  **(long-term)** | ***P. subcapitata*** |
| **AF** | | 100 | 100 | 10 | 10 |
|  | | **M650F01** | | | |
| Endpoint (µg/L) | | LC50 | EC50 | NOEC | ErC50 |
| - | > 100000 | - | - |
| RAC (µg/L) | | - | > 1000 | - | - |
| FOCUS | PEC gl-max (µg/L) 1) | PEC/RAC ratio | | | |
| Step 1 | 156.424 | - | < 0.16 | - | - |
|  | | **M650F03** | | | |
| Endpoint (µg/L) | | LC50 | EC50 | NOEC | ErC50 |
| > 82600 | > 82600 | 41750 | > 82600 |
| RAC (µg/L) | | > 826 | > 826 | 4175 | > 8260 |
| FOCUS | PEC gl-max (µg/L) 1) | PEC/RAC ratio | | | |
| Step 1 | 156.827 | < 0.19 | < 0.19 | 0.04 | < 0.02 |
|  | | **M650F04** | | | |
| Endpoint (µg/L) | | LC50 | EC50 | NOEC | ErC50 |
| > 100000 | > 100000 | - | > 100000 |
| RAC (µg/L) | | > 1000 | > 1000 | - | > 10000 |
| FOCUS | PEC gl-max (µg/L) 1) | PEC/RAC ratio | | | |
| Step 1 | 87.86 | < 0.09 | < 0.09 | - | < 0.01 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

1) Worst-case PEC value resulting from calculations for two-fold application.

For the intended uses in potatoes, fruiting vegetables and onions, calculated PEC/RAC ratios for the relevant metabolites of Ametoctradin indicate an acceptable risk for all groups of aquatic organisms based on FOCUS Step 1 PECSW calculations. Therefore, no further assessment is necessary for the relevant metabolites of Ametoctradin.

**Formulated product BAS 743 03 F**

**Table 9.5‑10: Aquatic organisms: Maximum RQmix for fish, aquatic invertebrates and algae based on the risk assessment of individual active substances**

|  |  |  |  |
| --- | --- | --- | --- |
| **Focus scenario** | **RQ Ametoctradin** | **RQ Propamocarb** | **RQmix** |
| **Acute fish** | | | |
| **FOCUS Step 1** | 0.09 | 0.50 | 0.59 |
| **FOCUS Step 2** | 0.01 | 0.10 | 0.11 |
| **Acute aquatic invertebrates** | | | |
| **FOCUS Step 1** | 0.08 | 0.46 | 0.54 |
| **FOCUS Step 2** | 0.01 | 0.09 | 0.10 |
| **Algae** | | | |
| **FOCUS Step 1** | 0.01 | 0.05 | 0.06 |
| **FOCUS Step 2** | 0.00 | 0.01 | 0.01 |

RQmix values below 1 for the worst-case FOCUS scenarios indicate an acceptable risk from combined exposure to Ametoctradin and Propamocarb.

**Table 9.5‑11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for BAS 743 03 F for each organism group based on drift entry for the use of BAS 743 03 F at 2 × 2.0 L/ha**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
| **Test species** | | ***O. mykiss \**** | ***D. magna \**** | ***P. subcapitata*** |
| **Endpoint [µg/L]** | | LC50 | EC50 | ErC50 |
| > 100000 | > 100000 | > 100000 |
| **AF** | | 100 | 100 | 10 |
| **RAC [µg/L]** | | > 1000 | > 1000 | > 10000 |
|  | **PECsw gl-max [µg/L]** | **PEC/ RAC ratio** | | |
| Drift (1 m) | 13.762  (Ditch) | <0.0138 | <0.0138 | <0.00138 |
| 0.469  (Pond) | <0.0005 | <0.0005 | <0.00005 |
| 10.213  (Stream) | <0.0102 | <0.0102 | <0.0010213 |

For the intended worst-case use in field crops (covering use in potato, tomato, aubergine and onion), calculated PEC/RAC ratios indicated an acceptable risk for all groups of aquatic organisms based on drift PECSW values without the need for mitigation measures.

### Overall conclusions

Overall, an acceptable risk to aquatic organisms is demonstrated for all intended uses of BAS 743 03 F without the need of mitigation measures.

|  |
| --- |
| **Review Comments**:  The submitted risk assessment has been accepted.  The relevant predicted environmental concentrations in water (PECsw) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PECsw values and the results of laboratory toxicity testing. The PECsw Step 1-2 (for ametoctradin, its metabolites and propamocarb) and Step 3 (for ametoctradin) were used.  BAS 743 03 F applications close to surface water pose acceptable risk to aquatic organisms without any mitigation measures. |

## Effects on bees (KCP 10.3.1)

Acute contact and oral toxicity studies on honeybees have been carried out with the active substances Ametoctradin (BAS 650 F) and Propamocarb. Full details of these studies are provided in the respective EU documents and the EU agreed endpoints are used for the risk assessment on honeybees.

Studies on the chronic toxicity to honeybees with Ametoctradin (tested with the solo-formulation BAS 650 00 F containing 200 g Ametoctradin/L, nominal) are available and have been previously submitted in support of the solo-formulation BAS 650 00 F. For details on these studies please refer to Appendix 2 and the Registration Report for BAS 650 00 F (Registration Report BAS 650 00 F, Part B Section 9, Core Assessment Central Zone prepared by the Netherlands, September 2018).

Studies on the chronic toxicity to honeybees with Propamocarb-HCl are available and have been previously submitted to support the renewal of Propamocarb-HCl (RAR Propamocarb, Vol. 3 – CP B9, June 2017).

Effects on bees of BAS 743 03 F were not evaluated as part of the EU assessment of Ametoctradin and Propamocarb. Therefore, new data on the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) submitted with this application are listed in Appendix 1 and summarised in Appendix 2. Since differences in co-formulants and/or their concentration between both formulations are considered minimal and both formulations are SC (suspension concentrates), it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission. In addition, several ecotoxicological bridging studies have been performed with the formulation BAS 743 03 F on aquatic invertebrates (*Daphnia magna*, BAS Doc ID 2022/2033730), adult honey bees (*Apis mellifera*, acute oral and contact, XXXX Doc ID 2022/2033729), non-target terrestrial arthropods (*Aphidius rhopalosiphi,* XXXX Doc ID 2022/2033732) and chronic earthworms (*Eisenia fetida*, XXXX Doc ID 2022/2033731) indicating no increased toxicity of BAS 743 03 F compared to BAS 743 02 F based on the content of active substance.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process, plus the endpoints for the formulation have been taken into account.

Table ‑ Endpoints and effect values for Ametoctradin relevant for the risk assessment for bees

| Species | Substance | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Apis mellifera* (adults) | Ametoctradin | acute oral, 48 h | **LD50 (48 h) > 111.5 µg/bee** | EFSA Journal 2012; 10(11):2921 2006/1012047 |
| *Apis mellifera* (adults) | Ametoctradin | acute contact, 48 h | **LD50 (48 h) > 100.0 µg/bee** | EFSA Journal 2012; 10(11):2921 2006/1012047 |
| *Apis mellifera* (adults) | Ametoctradin (conducted with BAS 650 00 F) | chronic, 10 d | LDD50 (10 d) = 10.2 µg a.s./bee/dayLC50 (10 d) = 0.254 g a.s./kg food | new study 2014/1111114  Not evaluated |
| *Apis mellifera* (larvae) | Ametoctradin (conducted with BAS 650 00 F) | repeated exposure, 22 d | LD50 (22 d) = 23.1 µg a.s./larva  NOED (22d) = 7.1 µg a.s./larva | new study 2014/1111115  Not evaluated |

**Bold** values are used in the risk assessment

Table ‑2 Endpoints and effect values for Propamocarb relevant for the risk assessment for bees

| Species | Substance | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Apis mellifera* (adults) | Propamocarb-HCL | acute oral, 48 h | **LD50 (48 h) > 84 µg/bee** | EFSA Journal 2006; 78, 1-80 |
| *Apis mellifera* (adults) | Propamocarb-HCL | acute contact, 48 h | **LD50 (48 h) > 100.0 µg/bee** | EFSA Journal 2006; 78, 1-80 |
| *Apis mellifera* (adults) | Propamocarb-HCL | acute oral, 48 h | LD50 (48 h) > 122.1 µg/bee | RAR Propamocarb, Vol. 3 – CP B9, June 2017  Not evaluated |
| *Apis mellifera* (adults) | Propamocarb-HCL | acute contact, 48 h | LD50 (48 h) > 109.0 µg/bee | RAR Propamocarb, Vol. 3 – CP B9, June 2017  Not evaluated |
| *Bombus terrestris* (adults) | Propamocarb-HCL | acute oral, 48 h | LD50 (48 h) > 198.7µg/bee | RAR Propamocarb, Vol. 3 – CP B9, June 2017  Not evaluated |
| *Apis mellifera* (adults) | Propamocarb-HCl | chronic, 10 d | LDD50 (10 d) > 85.68 µg a.s./bee/day | RAR Propamocarb, Vol. 3 – CP B9, June 2017  Not evaluated |

**Bold** values are used in the risk assessment

Table ‑3 Endpoints and effect values for BAS 743 03 F relevant for the risk assessment for bees

| Species | Substance | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Apis mellifera* (adults) | BAS 743 02 F\* | acute contact, 48 h | **LD50 (48 h) > 1000 µg/bee** | new study  2022/2033708 |
| *Apis mellifera* (adults) | BAS 743 02 F\* | acute oral, 48 h | LD50 (48 h) > 840 µg/bee | new study  2022/2033708 |
| *Apis mellifera* (adults) | BAS 743 03 F | acute contact, 48 h | LD50 (48 h) > 1000 µg/bee | new study  2022/2033729 |
| *Apis mellifera* (adults) | BAS 743 03 F | acute oral, 48 h | **LD50 (48 h) > 816 µg/bee** | new study  2022/2033729 |
| *Bombus terrestris* (adults) | BAS 743 02 F\* | acute contact, 48 h | LD50 (48 h) > 1000 µg/bee | new study  2022/2033711 |
| *Bombus terrestris* (adults) | BAS 743 02 F\* | acute oral, 48 h | LD50 (48 h) > 271.2 µg/bee | new study  2022/2033711 |
| *Apis mellifera* (adults) | BAS 743 02 F\* | chronic, 10 d | **LDD50 (10 d) = 78.6 µg product/bee/day**  NOEDD = 41.7 µg product/bee/day | new study  2022/2033709 |
| *Apis mellifera* (larvae) | BAS 743 02 F\* | repeated exposure, 22 d | **NOED (22 d) = 39.1 µg product/larva/day**  ED10 (22 d) = 44.5 µg/larva/day | new study  2022/2033710 |

**Bold** values are used in the risk assessment

\* Study was conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl

### Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process, plus the endpoints for the formulation have been taken into account.

### Risk assessment

The risk assessment has been performed according to SANCO/10329/2002 rev 2 final, since the new EFSA GD *“Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)”* (EFSA Journal 2013; 1187):3295) has not yet been adopted by the Standing Committee on Plants, Animals, Food and Feed.

The application of BAS 743 03 F is intended in potato, fruiting vegetables, minor crops ~~ornamentals~~ and onions. The risk assessment is based on the maximum single application rate ~~in potatoes~~ of 2.0 L/ha., as this use represents a worst-case (see Section 9 Chapter 9.1 for details).

#### Hazard quotients for bees

The risk to honeybees was assessed using the maximum single application rate and the LD50 values to calculate hazard quotients (HQ) for oral exposure (QHO) and contact exposure (QHC).

Table ‑4 First-tier assessment of the risk for bees due to the use of Ametoctradin as contained in BAS 743 03 F according to the proposed ~~worst-case~~ use~~s~~

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | Potatoes, onions, fruiting vegetables and minor crops | | |
| Active substance | Ametoctradin | | |
| Application rate (g a.s./ha) | 240 | | |
| Test design | LD50 (lab.) (µg a.s./bee) | Single application rate (g a.s./ha) | QHO, QHC criterion: QH ≤ 50 |
| Oral toxicity | > 111.5 | 240 | < 2.15 |
| Contact toxicity | > 100.0 | < 2.4 |

QHO, QHC: Hazard quotients for oral and contact exposure.

Table ‑5 First-tier assessment of the risk for bees due to the use of Propamocarb as contained BAS 743 03 F according to the proposed ~~worst-case~~ use~~s~~

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | Potatoes onions, fruiting vegetables and minor crops | | |
| Active substance | Propamocarb-HCL | | |
| Application rate (g a.s./ha) | 902 | | |
| Test design | LD50 (lab.) (µg a.s./bee) | Single application rate (g a.s./ha) | QHO, QHC criterion: QH ≤ 50 |
| Oral toxicity | > 84 | 902 | < 10.7 |
| Contact toxicity | > 100.0 | < 9.02 |

QHO, QHC: Hazard quotients for oral and contact exposure.

Table ‑6 First-tier assessment of the risk for bees due to the use of BAS 743 03 F according to the proposed ~~worst-case~~ use~~s~~

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | Potatoes onions, fruiting vegetables and minor crops | | |
| Product | BAS 743 03 F | | |
| Application rate (g/ha) | 2142 1) | | |
| Test design | LD50 (lab.) (µg/bee) | Single application rate (g/ha) | QHO, QHC criterion: QH ≤ 50 |
| Oral toxicity | > 816 | 2142 | < 2.63 |
| Contact toxicity | > 1000 | < 2.14 |

QHO, QHC: Hazard quotients for oral and contact exposure.

1) Taking into account a single application of 2.0 L product/ha and the density of BAS 743 03 F of 1.071 g/cm3.

The calculated hazard quotients for acute oral and acute contact exposure are below the trigger value of 50, hence the risk to bees from the proposed applications of BAS 743 03 F is acceptable.

In addition, data on chronic oral toxicity to adult honeybees and on oral toxicity to honeybee larvae for a similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) are available. These studies indicate low toxicity to bees and meet the current data requirements (Commission Regulation (EU) No. 283/2013 and 284/2013). However, under the current risk assessment scheme (SANCO/10329/2002) there is no requirement to conduct a risk assessment with these endpoints.

**The following is an assessment of the chronic risk to bees made by the Applicant**

**EFSA Guidance (2013, updated 2014)**

Upon zRMS request, the Applicant in the following provides risk assessments based on EFSA Guidance (2013, updated 2014[[6]](#footnote-6)). The Applicant would like to point out that the risk assessment methodology based on EFSA Guidance (2013) is not agreed at EU level. The Guidance is not noted (and never will, since a revised version of the guidance has been published in 2023) and should therefore, not be applied for decision-making. The Applicant is aware that regardless of the status of implementation of EFSA bee guidance, some Member States request assessments following EFSA Guidance (2013). However, this can be dealt with at national level, thus, there should not be a formal requirement to present EFSA (2013) in the Core document. The Applicant has included the assessment in the Core dossier in a comprehensive approach and as requested by the zRMS, but it should not be considered for decision-making at zonal level.

Risk assessments were conducted using the EFSA calculator tool (Bee-Tool, Version 3, 2020[[7]](#footnote-7)). Acute and chronic risk assessments are most appropriately presented based on the most relevant data from testing the representative formulation BAS 743 03 F and a similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) covering the combined exposure to the active substances. Acute risk assessments are also presented based on the available data for the active substances.

Acute and chronic assessments are presented for the major crops considered in the Core Assessment (i.e. potato, onion, tomato and aubergine) at the maximum single use rate of 2 L product/ha.

Screening Step assessments

**Table 9.6‑7: Screening Step assessment of the acute and chronic risk for bees due to the use of BAS 743 03 F in field crops (2 L/ha)**

| **Crop(s)** | **Maximum single use rate**  **[g/ha]** | **Species** | **Test item** | **Endpoint**  **[µg/bee]** | **Assessment** | **Calculation factor**  **(Ef × SV)** | **HQ / ETR** | **HQ / ETR trigger** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Field crops a) | 240 | HB | Ametoctradin | > 100.0 | Acute contact toxicity | n.a. | < 2.4 | ≤ 42 |
| 902 | HB | Propamocarb-HCl | > 100 | < 9.0 |
| 2142 b) | HB | BAS 743 03 F | > 1000 | < 2.1 |
| 240 | HB | Ametoctradin | > 111.5 | Acute oral toxicity | 7.6 | < 0.02 | ≤ 0.2 |
| 902 | HB | Propamocarb-HCl | > 84 | < 0.08 |
| 2142 b) | HB | BAS 743 03 F | > 816 | < 0.02 |
| HB | BAS 743 02 F\* | 78.6 | Chronic toxicity | 7.6 | **0.207** | ≤ 0.03 |
| HB | BAS 743 02 F\* | 39.1 | Bee larval toxicity | 4.4 | **0.24** | ≤ 0.2 |

HB: Honey bees; Ef: Exposure factor (= 1 for Screening Step for worst-case treated crop); SV: Shortcut Value; **bold**: HQ/ETR failing the assessment trigger for acceptability of risk.

a) Covering the intended worst-case uses in potatoes, onions and fruiting vegetables.

b) Based on an application rate of 2 L product/L and the density of BAS 743 03 F of 1.071 g/cm3.

\* Study was conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl.

Accordingly, based on Screening Step assessments, an acceptable acute risk is indicated for the intended uses.

Based on the data for the formulated product, potential chronic risk for adult honeybees and honeybee larvae cannot be excluded based on Screening Step assessments. In the following table, Tier 1 level chronic risk assessments are presented.

**Table 9.6‑8: Tier 1 assessment of the chronic risk for honey bees due to the uses of BAS 743 03 F**

| **Crop(s)** | **Maximum single use rate**  **[g/ha]** | **Test item** | **Endpoint**  **[µg a.s./bee]** | **Assessment** | **Scenario** | **BBCH** | **Ef** | **SV** | **TWA** | **ETR** | **ETR trigger** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Potatoes | 2142 a) | BAS 743 02 F\* | 78.6 | Chronic toxicity | Treated crop | 10-39 | 1 | 0.92 | 0.72 | 0.018 | ≤ 0.03 |
| 40-69 | 1 | 0.92 | 0.018 |
| ≥ 70 | 1 | 0 | 0.000 |
| Weeds | 10-39 | 1 | 2.9 | **0.057** |
| 40-69 | 0.3 | 2.9 | 0.017 |
| ≥ 70 | 0.3 | 2.9 | 0.017 |
| Field margin | 10-39 | 0.0092 | 2.9 | 0.001 |
| 40-69 | 0.0092 | 2.9 | 0.001 |
| ≥ 70 | 0.0092 | 2.9 | 0.001 |
| Adjacent crop | 10-39 | 0.0033 | 5.8 | 0.000 |
| 40-69 | 0.0033 | 5.8 | 0.000 |
| ≥ 70 | 0.0033 | 5.8 | 0.000 |
| Next crop | 10-39 | 1 | 0.54 | 0.011 |
| 40-69 | 1 | 0.54 | 0.011 |
| ≥ 70 | 1 | 0.54 | 0.011 |
| 39.1 | Bee larval toxicity | Treated crop | 10-39 | 1 | 0.15 | 0.85 | 0.01 | ≤ 0.2 |
| 40-69 | 1 | 0.15 | 0.01 |
| ≥ 70 | 1 | 0 | 0.00 |
| Weeds | 10-39 | 1 | 2.2 | 0.10 |
| 40-69 | 0.3 | 2.2 | 0.03 |
| ≥ 70 | 0.3 | 2.2 | 0.03 |
| Field margin | 10-39 | 0.0092 | 2.2 | 0.00 |
| 40-69 | 0.0092 | 2.2 | 0.00 |
| ≥ 70 | 0.0092 | 2.2 | 0.00 |
| Adjacent crop | 10-39 | 0.0033 | 4.4 | 0.00 |
| 40-69 | 0.0033 | 4.4 | 0.00 |
| ≥ 70 | 0.0033 | 4.4 | 0.00 |
| Next crop | 10-39 | 1 | 0.4 | 0.02 |
| 40-69 | 1 | 0.4 | 0.02 |
| ≥ 70 | 1 | 0.4 | 0.02 |
| Onions  (bulb vegetables) | 2142 a) | BAS 743 02 F\* | 78.6 | Chronic toxicity | Treated crop | 10-39 | 1 | 5.8 | 0.72 | **0.114** | ≤ 0.03 |
| 40-69 | 1 | 5.8 | **0.114** |
| Weeds | 10-39 | 1 | 2.9 | **0.057** |
| 40-69 | 0.6 | 2.9 | **0.034** |
| Field margin | 10-39 | 0.0092 | 2.9 | 0.001 |
| 40-69 | 0.0092 | 2.9 | 0.001 |
| Adjacent crop | 10-39 | 0.0033 | 5.8 | 0.000 |
| 40-69 | 0.0033 | 5.8 | 0.000 |
| Next crop | 10-39 | 1 | 0.54 | 0.011 |
| 40-69 | 1 | 0.54 | 0.011 |
| 39.1 | Bee larval toxicity | Treated crop | 10-39 | 1 | 4.4 | 0.85 | **0.20** | ≤ 0.2 |
| 40-69 | 1 | 4.4 | **0.20** |
| Weeds | 10-39 | 1 | 2.2 | 0.10 |
| 40-69 | 0.6 | 2.2 | 0.06 |
| Field margin | 10-39 | 0.0092 | 2.2 | 0.00 |
| 40-69 | 0.0092 | 2.2 | 0.00 |
| Adjacent crop | 10-39 | 0.0033 | 4.4 | 0.00 |
| 40-69 | 0.0033 | 4.4 | 0.00 |
| Next crop | 10-39 | 1 | 0.4 | 0.02 |
| 40-69 | 1 | 0.4 | 0.02 |
| Tomato and aubergine  (fruiting vegetables) | 2142 a) | BAS 743 02 F\* | 78.6 | Chronic toxicity | Treated crop | 10-49 | 1 | 0.92 | 0.72 | 0.018 | ≤ 0.03 |
| 50-69 | 1 | 0.92 | 0.018 |
| ≥ 70 | 1 | 0 | 0.000 |
| Weeds | 10-49 | 1 | 2.9 | **0.057** |
| 50-69 | 0.3 | 2.9 | 0.017 |
| ≥ 70 | 0.3 | 2.9 | 0.017 |
| Field margin | 10-49 | 0.0092 | 2.9 | 0.001 |
| 50-69 | 0.0092 | 2.9 | 0.001 |
| ≥ 70 | 0.0092 | 2.9 | 0.001 |
| Adjacent crop | 10-49 | 0.0033 | 5.8 | 0.000 |
| 50-69 | 0.0033 | 5.8 | 0.000 |
| ≥ 70 | 0.0033 | 5.8 | 0.000 |
| Next crop | 10-49 | 1 | 0.54 | 0.011 |
| 50-69 | 1 | 0.54 | 0.011 |
| ≥ 70 | 1 | 0.54 | 0.011 |
| 39.1 | Bee larval toxicity | Treated crop | 10-49 | 1 | 0.15 | 0.85 | 0.01 | ≤ 0.2 |
| 50-69 | 1 | 0.15 | 0.01 |
| ≥ 70 | 1 | 0 | 0.00 |
| Weeds | 10-49 | 1 | 2.2 | 0.10 |
| 50-69 | 0.3 | 2.2 | 0.03 |
| ≥ 70 | 0.3 | 2.2 | 0.03 |
| Field margin | 10-49 | 0.0092 | 2.2 | 0.00 |
| 50-69 | 0.0092 | 2.2 | 0.00 |
| ≥ 70 | 0.0092 | 2.2 | 0.00 |
| Adjacent crop | 10-49 | 0.0033 | 4.4 | 0.00 |
| 50-69 | 0.0033 | 4.4 | 0.00 |
| ≥ 70 | 0.0033 | 4.4 | 0.00 |
| Next crop | 10-49 | 1 | 0.4 | 0.02 |
| 50-69 | 1 | 0.4 | 0.02 |
| ≥ 70 | 1 | 0.4 | 0.02 |

SV: Shortcut Value; TWA: time-weighted average factor; Ef: Exposure factor; SV: Shortcut Value; **bold**: ETR failing the assessment trigger for acceptability of risk. \* Study was conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl.

a) Based on an application rate of 2 L product/L and the density of BAS 743 03 F of 1.071 g/cm3.

Based on the risk assessments as in accordance with EFSA (2013), risk can formally not be excluded for the following scenarios:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Crop(s)** | **Maximum single use rate**  **[g/ha]** | **Assessment** | **Scenario** | **BBCH** | **ETR** | **ETR trigger** | **Exceeding factor** |
| Potatoes | 2142 a) | Chronic toxicity | Weeds | 10-39 | 0.057 | ≤ 0.03 | 1.9 |
| Onions  (bulb vegetables) | Chronic toxicity | Treated crop | 10-39 | 0.114 | ≤ 0.03 | 3.8 |
| 40-69 | 0.114 | 3.8 |
| Weeds | 10-39 | 0.057 | 1.9 |
| 40-69 | 0.034 | 1.1 |
| Bee larval toxicity | Treated crop | 10-39 | 0.20 | ≤ 0.2 | 1.0 |
| 40-69 | 0.20 | 1.0 |
| Tomato and aubergine  (fruiting vegetables) | Chronic toxicity | Weeds | 10-49 | 0.057 | ≤ 0.03 | 1.9 |

a) Based on an application rate of 2 L product/L and the density of BAS 743 03 F of 1.071 g/cm3.

Refined risk assessment for chronic exposure of adults honey bee and honey bee larvae

In EFSA (2013), the default DT50 of 10 days is applied for pollen and nectar. However, in the recently published revised EFSA Guidance document on bee risk assessment (2023), default DT50 values of only 2 days for nectar and 3 days for pollen are proposed, based on a large database. Although the guidance is not in force yet, the review of the RUD data carried out to derive the above-mentioned DT50 values represents the state-of-the-art scientifically. Furthermore, the database was already published in a separate report in 2017 (Kyriakopoulou *et al*. [[8]](#footnote-8)) and only supplemented by four additional trials gathered by EFSA during the development of the 2023 guidance document (as stated in EFSA guidance 2023, Annex A of supplementary document). Thus, the DT50 values are considered applicable to the risk assessment for refinement and more relevant than the worst-case assumptions used in accordance with the obsolete EFSA Guidance of 2013.

According to EFSA (2013) and EFSA (2023) no pollen uptake is indicated to be relevant for forager bees, thus, for adult honey bees, a fTWA of 0.28 was calculated considering a 10-day time window and a DT50 of 2 days due to consumption of nectar only.

In contrast, honeybee larvae consume both, nectar and pollen. In a worst-case approach, the fTWA was calculated assuming only consumption of pollen. This is a highly conservative assumption as EFSA (2013) reports a small consumption of pollen (maximum 2.0 mg/larva) compared to the consumption of nectar (uptake of 59.4 mg sugar/larva with variable sugar content depending on the scenario). For bee larvae, a worst-case refined fTWA of 0.59 was calculated based on a DT50 of 3 days for pollen and a 5-day time window.

New ETR values are presented in the Table below applying the newly calculated fTWA.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Crop(s)** | **Maximum single use rate**  **[g/ha]** | **Assessment** | **Scenario** | **BBCH** | **ETR** | **ETR trigger** | **Exceeding factor** |
| Potatoes | 2142 a) | Chronic toxicity | Weeds | 10-39 | 0.022 | ≤ 0.03 | - |
| Onions  (bulb vegetables) | Chronic toxicity | Treated crop | 10-39 | 0.044 | ≤ 0.03 | 1.5 |
| 40-69 | 0.044 | 1.5 |
| Weeds | 10-39 | 0.022 | - |
| 40-69 | 0.013 | - |
| Bee larval toxicity | Treated crop | 10-39 | 0.142 | ≤ 0.2 | - |
| 40-69 | 0.142 | - |
| Tomato and aubergine  (fruiting vegetables) | Chronic toxicity | Weeds | 10-49 | 0.022 | ≤ 0.03 | - |

a) Based on an application rate of 2 L product/L and the density of BAS 743 03 F of 1.071 g/cm3.

ETR values for the chronic risk to honeybee larvae are below the trigger for all relevant exposure scenarios based on the refined fTWA (worst-case), indicating an acceptable risk for honeybee larvae. Likewise, ETR values for the chronic risk to adult honeybees are below the trigger except for the treated crop scenario which slightly exceed the trigger for single application of 2 L product/ha in onions. Considering that onion crops are usually harvest before flowering, exposure from foraging on the treated crop can be considered negligible.

Overall, an acceptable risk is indicated based on the DT50 refinements. Even if ETR values for adult honeybees are above the trigger for the treated crop scenario in onions, the exceedance is very small (i.e. factor of 1.5) and exposure is considered negligible due to the common agricultural practise for this crop of harvesting pre-flowering. Therefore, the risk for bees according to the intended uses is acceptable.

#### Higher-tier risk assessment fr bees (tunnel test, field studies)

Not required.

### Effects on bumble bees

Effects on bumble bees are not a data requirement under Commission Regulation (EU) No. 283/2013 and 284/2013 and are not included in the current risk assessment scheme under SAN-CO/10329/2002. Furthermore, the EFSA Technical Report on the “Outcome of the pesticide peer review meeting on general recurring issues in ecotoxicology” (EFSA Supporting publication 2015:EN-924) states that it cannot be recommended to routinely perform risk assessments for bumble bees. Therefore, a risk assessment on bumble bees has not been conducted. However, data for bumble bees from testing with the formulated product are available. The limit dose endpoints for the product, as well as the available limit dose endpoints for the active substance Propamocarb-HCl do not suggest higher sensitivities towards the formulated product as compared to honeybees.

### Effects on solitary bees

Effects on solitary bees are not a data requirement under Commission Regulation (EU) No. 283/2013 and 284/2013 and are not included in the current risk assessment scheme under SAN-CO/10329/2002. Furthermore, the EFSA Technical Report on the “Outcome of the pesticide peer review meeting on general recurring issues in ecotoxicology” (EFSA Supporting publication 2015:EN-924) states that it cannot be recommended to routinely perform risk assessments for solitary bees. Therefore, a risk assessment on solitary bees has not been conducted.

### Overall conclusions

The hazard quotients for BAS 743 03 F and the active substances Ametoctradin and Propamocarb for acute oral and acute contact exposure of honeybees are considerably below the Commission Regulation (EU) 546/2011 trigger value of 50, indicating an acceptable risk. Based on these results it can be concluded that a low risk to honeybees is expected from applications of BAS 743 03 F according to the proposed uses.

|  |
| --- |
| **Review Comments:**  The evaluation of the acute risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to BAS 743 03 F.  The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.  Some CEU MSs countries require evaluation according to EFSA 2013. This approach is still not harmonised, but it was discussed at the last meeting of the central zone in the field of ecotoxicology (Warsaw, 12.2023), where it was agreed to present an assessment in the Core in accordance with EFSA 2013. Thus, required chronic risk assessment was provided. ~~Currently, the minutes of the meeting are still in the course of zonal consultations.~~ For Poland, a chronic risk assessment is not required. ~~Nevertheless, the Applicant, as a result of commenting process, may be asked to supplement the dossier with a risk assessment for bees in accordance with EFSA 2013.~~ |

## Effects on arthropods other than bees (KCP 10.3.2)

### Toxicity data

Studies on the toxicity to non-target arthropods have not been carried out with technical active substances Ametoctradin and Propamocarb. As standard practice the risk to non-target arthropods is based on formulation specific data.

Effects on non-target arthropods of BAS 743 03 F were not evaluated as part of the EU assessment of the active substances.

The toxicity of BAS 743 03 F to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. New data submitted with this application are listed in Table 9.7‑1, Appendix 1 and summarized in Appendix 2. Since differences in co-formulants and/or their concentration between both formulations are considered minimal, it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission. In addition, the toxicity of BAS 743 03 F has been investigated in a Tier I test on *Aphidius rhopalosiphi* (BAS Doc ID 2022/2033732). indicating no increased toxicity of BAS 743 03 F compared to BAS 743 02 F based on the content of active substance.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table ‑ Endpoints and effect values for BAS 743 03 F relevant for the risk assessment for non-target arthropods

| Species | Product | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| Laboratory tests | | | | |
| *Aphidius rhopalosiphi* (adults) | BAS 743 02 F | Laboratory test glass plates 2D exposure | LR50 > 3 L/ha  ER50 > 3 L/ha | new study  2022/2033728 |
| *Typhlodromus pyri*  (protonymphs) | BAS 743 02 F | ~~Extended~~ laboratory test ~~bean leaf discs,~~ 2D exposure | LR50 > 3 L/ha  ER50 > 3 L/ha | 2022/2033725 |
| *Aphidius rhopalosiphi* (adults) | BAS 743 03 F | Laboratory test glass plates 2D exposure | LR50 > 4.6 L/ha  ER50 > 4.6 L/ha | new study  2022/2033732 |

#### Justification for new endpoints

Effects of BAS 743 03 F on non-target arthropods other than bees are most appropriately based on data for the formulated product. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

### Risk assessment

The testing and risk assessment strategy used here follow the approaches recommended in the ESCORT 2 guidance document, and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002)*.* The risk assessment for BAS 743 03 F is based on tests with the standard test species *Typhlodromus pyri* and *Aphidius rhopalosiphi.*

#### Risk assessment for in-field exposure

Non-target arthropods living in the crop can be exposed to residues from direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. The maximum in-field exposure (predicted environmental rate, PER) is calculated according to ESCORT 2. As a pre-emergence or early post-emergence application is not intended for the use of BAS 743 03 F (see Section 9 Chapter 9.1 for details), the PER (soil) will not be considered in the following risk assessment.

Table ‑: In-field predicted environmental rates (PER) for BAS 743 03 F for all uses

|  |  |  |
| --- | --- | --- |
| **Substance** | **Application rate** | **in-field PER (foliar)** |
| **Potato 3 x 2.0 L product/ha (covering uses in other field crops, including, fruiting vegetables)** | | |
| BAS 743 03 F | 3 x 2.0 L product/ha | 4.6 L product/ha  (MAF = 2.3) |
| **Onion 2 x 2.0 L product/ha** | | |
| BAS 743 03 F | 2 x 2.0 L product/ha | 3.4 L product/ha  (MAF = 1.7) |

MAF: Multiple application factor, PER: Predicted environmental

The details of the first-tier risk assessments for the proposed worst-case crops are presented in the following table.

Table ‑3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the worst-case use of BAS 743 03 F in field crops at 3 x 2.0 L/ha

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | Field crops | | |
| Product | BAS 743 03 F | | |
| Application rate (L/ha) | 3 x 2.0 L product/ha | | |
| MAF | 2.3 (vegetation) | | |
| Test species | **Tier I** | | |
| LR50 (lab.) [L/ha] | PERin‑field [L/ha) | HQin-field criterion: HQ ≤ 2 |
| *Typhlodromus pyri* | > 3.0 # | 4.6 | 1.5 |
| *Aphidius rhopalosiphi* | > 3.0 # | 1.5 |

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient.

# Dose rate with 50% effect not determined as no effect was observed at any tested dose rate

An acceptable in-field risk for *Typhlodromus pyri* and *Aphidius rhopalosiphi,* is indicated based on glass plate (first tier) laboratory data. Therefore, an overall acceptable risk to non-target arthropods from the intended uses of BAS 743 03 F can be concluded.

#### Risk assessment for off-field exposure

Exposure of non-target arthropods living in off-field areas to BAS 743 03 F will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure via soil residues in off-field areas was not considered. Off-field foliar PER values were calculated for the worst-case proposed uses, from in-field foliar PER values in conjunction with drift values listed in Appendix IV of the ESCORT 2 guidance document (Table 9.7-5).

A vegetation distribution or dilution factor is included in the equation when calculating PER values from toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 5 is conservatively applied in line with the EFSA Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (2019).

Table ‑4: PERoff-field values following worst-case applications of BAS 743 03 F

| **Study type (Exposure scenario)** | **Maximum PERin-field [L/ha]** | **Drift factor [% drift/100]** | **Vegetation distribution factor\*** | **PERoff-field [L/ha]** |
| --- | --- | --- | --- | --- |
| **Potatoes** | | | | |
| 2D | 4.6 | 0.0201 a | 5 | 0.018 |

\* As recommended in EFSA Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (2019).

a Drift value for three applications in field crops (82nd percentile)

In order to assess the potential risk of BAS 743 03 F to off-field non-target arthropods, the PERoff-field (see Table 9.7‑) is compared to the toxicity endpoints.

ESCORT 2 recommends a correction factor of 10 for Tier I data in the off-field risk assessment to account for extrapolation from testing just few representative species to the species diversity expected in off-crop areas.

Table ‑5: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the worst-case use of BAS 743 03 F at in field crops 2 x 2.0 L a.s./ha

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Intended use | Field crops | | | | |
| Product | BAS 743 03 F | | | | |
| Application rate (L/ha) | 3 x 2.0 L product/ha | | | | |
| MAF | 2.3 (vegetation) | | | | |
| vdf | 5 (2D exposure) / - (3D exposure) | | | | |
| Test species | **Tier I** | | | | |
| LR50 (lab.) [L/ha] | Drift rate (%) | PERoff‑field [L/ha] | CF | HQoff-field criterion:  HQ ≤ 2 |
| *Typhlodromus pyri* | > 3.0 | 2.01 | 0.018 | 10 | 0.06 |
| *Aphidius rhopalosiphi* | > 3.0 | 0.06 |

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

An acceptable off-field risk for *Typhlodromus pyri* and *Aphidius rhopalosiphi* was found based on glass plate laboratory data. Therefore, an overall acceptable risk to non-target arthropods from the intended uses of BAS 743 03 F can be concluded.

#### Additional higher-tier risk assessment

Not required.

#### Risk mitigation measures

No risk mitigation needed.

### Overall conclusions

Based on the results of the conducted first and higher tier risk assessment it can be concluded that a low risk for non-target arthropods is expected from the use of BAS 743 03 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

|  |
| --- |
| **Review Comments:**  The submitted risk assessment for applications in field crops has been accepted.  Based on the results of the conducted risk assessment it can be concluded that no in-field and off-field risk for non-target arthropods is expected from use of BAS 743 03 F. No mitigation measures are required. |

## Effects on non-target soil meso- and macrofauna (KCP 10.4)

### Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) with Ametoctradin (tested with the solo-formulation BAS 650 00 F containing 200 g Ametoctradin/L, nominal) are available and have been previously submitted in support of the solo-formulation   
BAS 650 00 F. For details on these studies please refer to Appendix 2 and the Registration Report for BAS 650 00 F (Registration Report BAS 650 00 F, Part B Section 9, Core Assessment Central Zone prepared by the Netherlands, September 2018). Studies on the chronic toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with relevant Ametoctradin soil metabolites M650F03 and M650F04. Full details of these studies are provided in the respective EU DARs and related documents.

No chronic studies on the toxicity of Propamocarb-HCl to earthworms and other non-target soil organisms (meso- and macrofauna) were carried out during the EU peer review of the active substance. For the first Annex I approval of Propamocarb a study on the long-term toxicity to earthworms was conducted with the representative product Propamocarb-HCl SL722 (analysed content of Propamocarb: 727 g/L) and it was accepted at EU level that effects of the active substance on non-target organisms could be adequately assessed on the basis of the available data on the representative product (EFSA Scientific Report (2006) 78, 1-8). Studies on the toxicity to other non-target soil organisms (meso- and macrofauna) with Propamocarb-HCl are available and have been previously submitted to support the renewal of Propamocarb-HCl (RAR Propamocarb, Vol. 3 – CP B9, June 2017).

An overview of the toxicity endpoints for both active substances and their relevant soil metabolites are presented below (see Tables 9.8‑1 and 9.8-2).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of the new formulation BAS 743 03 F were not evaluated as part of the EU assessment of Ametoctradin or Propamocarb. New data are listed in Table 9.8-3 and Appendix 1 and are summarized in Appendix 2. Chronic toxicity testing of BAS 743 03 F has been performed with the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl) on earthworms (*Eisenia fetida*, XXXX Doc ID 2022/2033719) and other non-target soil marco-organisms (*Folsomia candida,* XXXX Doc ID 2022/2033720 and *Hypoaspis aculeifer*, XXXX Doc ID 2022/2033721). Since differences in co-formulants and/or their concentration between both formulations are considered minimal, it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission. In addition, a chronic study on earthworms (*Eisenia fetida*, XXXX Doc ID 2022/2033731) has been conducted with BAS 743 03 F indicating no increased toxicity of BAS 743 03 F compared to BAS 743 02 F based on the content of active substance.

Table 9.8‑1 Ametoctradin: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

| **Species** | **Substance** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| **Earthworms** | | | | |
| *Eisenia fetida* | Ametoctradin (tested as BAS 650 00 F) | Mixed into substrate 56 d, chronic 5% peat content | NOEC ≥ 20.5 mg a.s./kg dry soil  **NOECCORR ≥ 10.25 mg a.s./kg dry soil** | new study 2007/1037733  Not evaluated |
| *Eisenia fetida* | M650F03 | Mixed into substrate 56 d, chronic 10% peat content | **NOEC ≥ 83.5 mg/kg dry soil** | EFSA Journal 2012; 10(11):2921 2008/1010610 |
| *Eisenia fetida* | M650F04 | Mixed into substrate 56 d, chronic 10% peat content | **NOEC ≥ 100 mg/kg dry soil** | EFSA Journal 2012; 10(11):2921 2008/1010609 |
| **Other soil macro-organisms** | | | | |
| *Folsomia candida* | Ametoctradin (tested as BAS 650 00 F) | Mixed into substrate 28 d, chronic 5% peat content | NOEC ≥ 190.7 mg a.s./kg dry,  **NOECCORR > 95.3 mg a.s./kg dry soil** | new study 2007/1037734  Not evaluated |
| *Hypoaspis aculeifer* | Ametoctradin (tested as BAS 650 00 F) | Mixed into substrate 14 d, chronic 5% peat content | NOEC ≥ 190.7 mg a.s./kg dry soil,  EC10 > 190.7 mg a.s./kg dry soil  **NOECCORR > 95.3 mg a.s./kg dry soil**  EC10 CORR > 95.3 mg a.s./kg dry soil | new study 2016/1193035  Not evaluated |
| *Folsomia candida* | M650F03 | Mixed into substrate 28 d, chronic 10% peat content | **NOEC = 50.0 mg/kg dry soil** | EFSA Journal 2012; 10(11):2921 2008/1032646 |
| *Folsomia candida* | M650F04 | Mixed into substrate 28 d, chronic 10% peat content | **NOEC ≥ 100 mg/kg dry soil** | EFSA Journal 2012; 10(11):2921 2008/1032647 |
| *Hypoaspis aculeifer* | M650F03 | Mixed into substrate 14 d, chronic 5% peat content | **NOEC ≥ 100 mg/kg dry soil** | EFSA Journal 2012; 10(11):2921 2008/1031478 |
| *Hypoaspis aculeifer* | M650F04 | Mixed into substrate 14 d, chronic 5% peat content | **NOEC ≥ 100 mg/kg dry soil** | EFSA Journal 2012; 10(11):2921 2008/1031479 |

\* As the log Pow of ametoctradin is > 2, the EPPO correction factor of 2 has been applied to the active substance endpoints. The metabolites M650F03 and M650F04 have a log Pow of 0.2, therefore the EPPO correction does not need to be applied.

**Bold** figures: Endpoint used in aquatic risk assessment.

**Table 9.8‑2 Propamocarb-HCl: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)**

| **Species** | **Substance** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| **Earthworms** | | | | |
| *Eisenia fetida* | Propamocarb-HCl (tested as Propamocarb-HCl SL 722) | 56 d, chronic, sprayed | **NOEC ≥ 362 mg a.s./kg dry soil** | EFSA Scientific Report (2006) 78, 1-80 |
| **Other soil macro-organisms** | | | | |
| *Folsomia candida* | Propamocarb-HCl (tested as Propamocarb-HCl SL 722) | Mixed into substrate 28 d, chronic 5% peat content | **NOEC ≥ 677 mg a.s./kg dry,** | RAR Propamocarb, Vol. 3 – CP B9, June 2017  Not evaluated |
| *Hypoaspis aculeifer* | Propamocarb-HCl (tested as Propamocarb-HCl SL 722) | Mixed into substrate 14 d, chronic 5% peat content | **NOEC ≥ 677 mg a.s./kg dry soil** | RAR Propamocarb, Vol. 3 – CP B9, June 2017  Not evaluated |

**Table 9.8‑3: BAS 743 03 F: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Substance** | **Exposure System** | **Results\*** | **Reference** |
| **Chronic** | | | | |
| *Eisenia andrei* | BAS 743 02 F\*\* | Mixed into substrate 56 d, chronic 10% peat content | NOEC = 171 mg product/kg dry soil  **NOECCORR = 85.5 mg product/kg dry soil**  EC10 = n.d. | new study 2022/2033719 |
| *Eisenia andrei* | BAS 743 03 F | Mixed into substrate 56 d, chronic 10% peat content | NOEC = 171 mg product/kg dry soil  **NOECCORR = 85.5 mg product/kg dry soil**  EC10 = n.d. | new study 2022/2033731 |
| *Folsomia candida* | BAS 743 02 F\*\* | Mixed into substrate 28 d, chronic 5% peat content | NOEC ≥ 556 mg product/kg dry soil  **NOECCORR > 278 mg product/kg dry soil**  EC10 n.d. | new study 2022/2033720 |
| *Hypoaspis aculeifer* | BAS 743 02 F\*\* | Mixed into substrate 14 d, chronic 5% peat content | NOEC ≥ 1000 mg product/kg dry soil  **NOECCORR > 500 mg product/kg dry soil**  EC10 > 1000 mg product/kg dry soil | new study 2022/2033721 |

\* As the log Pow of ametoctradin is > 2, the EPPO correction factor of 2 has been applied.

\*\* Studies were conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl

n.d. not determined

**Bold** figures: Endpoint used in aquatic risk assessment.

#### Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process, plus new endpoints for Ametoctradin (tested as BAS 650 00 F) and for the formulations BAS 743 02 F and BAS 743 03 F have been taken into account as the endpoints for the formulated product are most relevant for the mixture of active substances.

Commission Regulation (EU) 283/2013 and (EU) 284/2013 require estimates of ECX values (e.g. EC10, EC20) together with the NOEC value as toxicity endpoints for the risk-assessment on non-target soil meso- and macrofauna. Where possible, ECx values were calculated. However, since in all cases the NOEC values were lower that the EC10 values, the NOECs were used in the risk assessment.

### Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

#### First-tier risk assessment

The relevant predicted environmental concentrations in soil (PECsoil) for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-4 ff. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for the active substance ametoctradin and its metabolite M650F03. In contrast, multi-annual accumulation needs to be considered for the Ametoctradin metabolite M650F04 and for Propamocarb-HCl. Following the EFSA Journal 2012;10(11):2921, no assessment was performed for the Ametoctradin metabolites M650F01 and M650F02.

BAS 74303 F is applied at a maximum rate of 3 × 2.0 L/ha in potatoes, 2 × 2.0 L/ha in tomatoes and aubergine and 2 × 2.0 L/ha in onion. Following SANCO/6895/2009 rev 1, all uses required across the zone are covered within the present core assessment. The worst-case PECsoil values which cover all uses relevant for the risk assessment (*i.e.* derived from the use in onion for the active substance Ametoctradin and for the metabolite M650F03 as well as from the use in cucurbits for the Ametoctradin metabolite M650F04 and for Propamocarb-HCL) were calculated as described in Chapter 8.7.2.

For substances with log POW values > 2 and studies with an artificial soil, the resulting endpoints should be corrected by a factor of 2 (foc) in the risk assessment in order to address lower contents of organic material in natural soil, unless it can be demonstrated by soil sorption data or other evidence that the toxicity is independent of foc. Based on log POW of 4.4 for Ametoctradin, a correction needs to be applied. The corrected values in Table 9.8-4 are indicated as NOECCORR. The metabolites M650F03 and M650F04 have a log Pow of 0.2 and 0.9, respectively, thus correction for organic matter is not necessary.

The potential risk of Ametoctradin, Propamocarb-HCl, their relevant metabolites and BAS 743 03 F to earthworms and other non-target soil macro-organisms was assessed by comparing the maximum PECsoil values with the relevant chronic endpoint (NOEC or EC10 values) to generate long-term TER values (TERlt), as presented in the following tables.

**Table 9.8‑4: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the worst-case uses of Ametoctradin in BAS 743 03 F**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | **Various crops (1 - 3 × 240 g a.s./ha)** | | |
| **Chronic effects on earthworms** | | | |
| A**ctive substance**/**metabolites** | **NOEC/NOECCORR (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| ~~Ametoctradin~~ | ~~≥ 10.25~~ | ~~0.389\*~~ | ~~30.33~~ |
| M650F03 | ≥ 83.5 | 0.243\*\* | ≥ 343.6 |
| M650F04 | ≥ 100 | ~~0.435~~~~\*~~ 0.280\*\* | ≥ ~~245.7~~ 357.1 |
| **Chronic effects on other soil meso- and macrofauna** | | | |
| **Collembola (*Folsomia candida*)** | | | |
| A**ctive substance/metabolites** | **NOEC/NOECCORR (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| ~~Ametoctradin~~ | ~~95.35~~ | ~~0.389\*~~ | ~~282.1~~ |
| M650F03 | 50 | 0.243\*\* | 205.8 |
| M650F04 | ≥ 100 | ~~0.435~~~~\*~~ 0.280\*\* | ≥ ~~245.7~~ 357.1 |
| **Soil mites (*Hypoaspis aculeifer*)** | | | |
| **Active substance/metabolites** | **NOEC/NOECCORR (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| ~~Ametoctradin~~ | ~~95.35~~ | ~~0.389\*~~ | ~~282.1~~ |
| M650F03 | ≥ 100 | 0.243\*\* | 411.5 |
| M650F04 | ≥ 100 | ~~0.435~~~~\*~~ 0.280\*\* | ≥ ~~245.7~~ 357.1 |

\* ~~PEC~~~~soil,~~ ~~in perennial crops~~~~representing the worst-case for Ametoctradin and metabolite M650F04~~

\*\* PECsoil, in onionrepresenting the worst-case for ~~Ametoctradin and~~ metabolite M650F03 and M650F04

**Table 9.8‑5: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of Propamocarb-HCl in BAS 743 03 F**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | **Various crops (1 - 3 × 902 g a.s./ha)** | | |
| **Chronic effects on earthworms** | | | |
| A**ctive substance**/**metabolites** | **NOEC/NOECCORR (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| Propamocarb-HCl | ≥ 362 | ~~2.399~~ 2.241\* | ≥ ~~150.9~~ 161.5 |
| **~~Chronic effects on other soil meso- and macrofauna~~** | | | |
| **~~Collembola (~~*~~Folsomia candida~~*~~)~~** | | | |
| ~~A~~**~~ctive substance/metabolites~~** | **~~NOEC/NOEC~~~~CORR~~ ~~(mg/kg dry soil)~~** | **~~PEC~~~~soil~~ ~~(mg/kg dry soil)~~** | **~~TER~~~~lt~~ ~~(criterion TER ≥ 5)~~** |
| ~~Propamocarb-HCl~~ | ~~≥ 677~~ | ~~2.399~~ | **~~≥~~** ~~282.2~~ |
| **~~Soil mites (~~*~~Hypoaspis aculeifer~~*~~)~~** | | | |
| **~~Active substance/metabolites~~** | **~~NOEC/NOEC~~~~CORR~~ ~~(mg/kg dry soil)~~** | **~~PEC~~~~soil~~ ~~(mg/kg dry soil)~~** | **~~TER~~~~lt~~ ~~(criterion TER ≥ 5)~~** |
| ~~Propamocarb-HCL~~ | ~~≥ 677~~ | ~~2.399~~ | ~~≥ 282.2~~ |

\* PECsoil, in perennial cropsrepresenting the worst-case for Propamocarb-HCl

As all TER values for both active substances and their metabolites are above the respective trigger value, the risk to earthworms and other soil meso- and macrofauna is considered to be acceptable for all intended uses of BAS 743 03 F.

**Table 9.8‑6: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the worst-case uses of BAS 743 03 F**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | **Various crops (1 -3 × 2 L/ha)** | | |
| **Chronic effects on earthworms** | | | |
| **Product** | **NOECCORR (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)\*** | **TERlt (criterion TER ≥ 5)** |
| BAS 743 03 F | 85.5 | ~~2.57~~ 7.711 | ~~33.27~~ 11.09 |
| **Chronic effects on other soil meso- and macrofauna** | | | |
| **Collembola (*Folsomia candida*)** | | | |
| **Product** | **NOECCORR (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)\*** | **TERlt (criterion TER ≥ 5)** |
| BAS 743 03 F | > 278 | ~~2.57~~ 7.711 | ≥ ~~108.2~~ 36.05 |
| **Soil mites (*Hypoaspis aculeifer*)** | | | |
| **Product** | **NOECCORR (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)\*** | **TERlt (criterion TER ≥ 5)** |
| BAS 743 03 F | > 500 | ~~2.57~~ 7.711 | ≥ ~~194.6~~ 64.84 |

As all TER values are above the respective trigger value, the risk to earthworms and other soil meso- and macrofauna is considered to be acceptable for all intended uses of BAS 743 03 F.

#### Higher-tier risk assessment

Not relevant.

### Overall conclusions

All TER values for BAS 743 03 F, the active substances Ametoctradin and Propamocarb, and relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. Therefore, it can be concluded that BAS 743 03 F poses no unacceptable risk to earthworms or other soil meso- and macrofauna.

|  |
| --- |
| **Review Comments:**  The long-term risks of BAS 743 03 F to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PECsoil. The relevant predicted environmental concentrations in soil (PECsoil) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).  Based on performed risk assessment it can be concluded that there will be negligible risk associated with the exposure of beneficial soil organisms to BAS 743 03 F following proposed use pattern. |

## Effects on soil microbial activity (KCP 10.5)

### Toxicity data

Studies on effects to soil micro-organisms have been carried out with the active substance Ametoctradin (tested as BAS 655 00 F containing 200 g ametoctradin/L, nominal) and its relevant soil metabolites. No studies on effects to soil micro-organisms have been carried out with the active substance Propamocarb-HCL during the EU peer review. For the first Annex I approval of Propamocarb, a study on effects to soil micro-organisms was conducted with the representative product Propamocarb-HCL SL722 (analysed content of Propamocarb: 727 g/L) and it was accepted at EU level that effects of the active substance on non-target organisms could be adequately assessed based on the available data on the representative product (EFSA Scientific Report (2006) 78, 1-8). Full details of these studies are provided in the respective EU DAR and related documents.

An overview of the toxicity endpoints for both active substances and relevant soil metabolites are presented below (see Table 9.9 1 and 9.9-2).

Effects on soil micro-organisms of BAS 743 03 F were not evaluated as part of the EU assessment of the active substances. New data submitted with this application are listed in Table 9.9-3 and Appendix 1 and summarised in Appendix 2. Effects on N-mineralisation of BAS 743 03 F have been tested with the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl). Since differences in co-formulants and/or their concentration between both formulations are considered minimal, it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission. In addition, several ecotoxicological bridging studies have been performed with the formulation BAS 743 03 F on aquatic invertebrates (*Daphnia magna*, BAS Doc ID 2022/2033730), adult honey bees (*Apis mellifera*, acute oral and contact, XXXX Doc ID 2022/2033729), non-target terrestrial arthropods (*Aphidius rhopalosiphi,* XXXX Doc ID 2022/2033732) and chronic earthworms (*Eisenia fetida*, XXXX Doc ID 2022/2033731) indicating no increased toxicity of BAS 743 03 F compared to BAS 743 02 F based on the content of active substance.

Table ‑: Endpoints and effect values of ametoctradin (tested as BAS 650 00 F) and relevant soil metabolites relevant for the risk assessment for soil micro-organisms

| Endpoint | Substance | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| N-mineralisation | Ametoctradin (tested as BAS 650 00 F) | 28 d, aerobic loamy sand | Nitrate formation rate 3.84 mg a.s./kg dry soil 0.7% | EFSA Journal 2012;10(11):2921  2006/1026123  + 2009/1075782 |
| M650F03 Reg. no. 5 178 870 | 28 d, aerobic sandy loam | Nitrate formation rate 8.02 mg/kg dry soil -2.7% | EFSA Journal 2012;10(11):2921 2008/1033292  + 2009/1075790 |
| M650F04 Reg. no. 5 211 623 | 28 d, aerobic sandy loam | Nitrate formation rate 13.4 mg/kg dry soil 4.6% | EFSA Journal 2012;10(11):2921 2008/1033293 + 2009/1075792 |

**Table 9.9‑2: Endpoints and effect values of Propamocarb-HCL relevant for the risk assessment for soil microorganisms**

| Endpoint | Substance | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| N-mineralisation | Propamocarb (tested as Propamocarb-HCl SL 722) | 28 d | No adverse effects  ≥ 28.9 mg a.s./kg dws | EFSA Scientific Report (2006) |

Table 9.9‑3: Endpoints and effect values of BAS 743 03 F relevant for the risk assessment for for soil micro-organisms

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Substance** | **Exposure System** | **Results** | **Reference** |
| **Chronic** | | | | |
| N-mineralisation | BAS 743 02 F\* | Mixed into substrate 28 d, chronic 10% peat content | < 25 % effect at day 28 at 25.20 mg/kg dry soil | new study 2022/2033717 |

#### \* Study was conducted with the similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl

#### Justification for new endpoints

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process, plus the endpoints for the formulation have been taken into account as this endpoint is most relevant for the mixture of the active substances.

### Risk assessment

The evaluation of the risk for soil micro-organisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant predicted environmental concentrations in soil (PECsoil) for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see Section 9 Chapter 9.8).

Table ‑4: Assessment of the risk for effects on soil micro-organisms due to the worst-case uses of BAS 743 03 F, ametoctradin and its relevant soil metabolites

|  |  |  |  |
| --- | --- | --- | --- |
| Intended use | **1 – 2 x 2.0 L BAS 743 03 F/ha in various crops** | | |
| N-mineralisation | | | |
| Active substance/metabolites | Max. conc. with effects ≤ 25 % (mg/kg dry soil) | PECsoil (mg/kg dry soil) | Risk acceptable? |
| Ametoctradin (tested as BAS 650 00 F) | > 3.84 (at 28 d) | ~~0.338\*~~ 0.386 | yes |
| M650F03 Reg. no. 5 178 870 | > 8.02 (at 28 d) | 0.243\*\*\* | yes |
| M650F04 Reg. no. 5 211 623 | > 13.4 (at 28 d) | ~~0.407~~ 0.280 | yes |
| Propamocarb-HCL | > 28.9 (at 28 d) | ~~2.399~~ 2.241 | yes |
| **Product** | **Max. conc. with effects ≤ 25 % (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **Risk acceptable?** |
| BAS 743 03 F | > 25.20 (at 28 d) | ~~2.57~~ 7.711\*\* | yes |

\* ~~PEC~~~~soil~~~~, in ornamentals (perennial crops) representing the worst-case for Ametoctradin, Propamocarb-HCl and the Ametoctradin metabolite M650F04~~

\*\*PECsoil, in field cropsrepresenting the worst-case for the formulation BAS 743 03 F

\*\*\* PECsoil, accu. in onionrepresenting the worst-case for Propamocarb-HCl and the Ametoctradin metabolite M652F03

No effects of >25% on soil nitrification were observed in tests with ametoctradin, the formulated product or the potentially relevant soil metabolites at concentrations higher than the maximum calculated PECsoil values. The risk to soil micro-organisms is therefore acceptable.

### Overall conclusions

For the formulation BAS 743 03 F, the active substance Ametoctradin, the relevant metabolites and the active substance Propamocarb-HCL, the maximum concentrations with effects < 25% (SANCO/10329/2002 trigger) are all by far above the maximum calculated PECsoil values. Therefore, it can be concluded that the use of BAS 743 03 F will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.

|  |
| --- |
| **Review Comments:**  Based on the results of the conducted first tier risk assessment it can be concluded that no risk for soil micro-organisms is expected from use of BAS 743 03 F. |

## Effects on non-target terrestrial plants (KCP 10.6)

### Toxicity data

Studies on the toxicity to non-target terrestrial plants have not been carried out with technical active substances Ametoctradin and Propamocarb. As standard practice the risk to non-target plants is based on formulation specific data.

Effects on non-target terrestrial plants of BAS 743 03 F were not evaluated as part of the EU assessment of Ametoctradin and Propamocarb. New data on the similar formulation BAS 743 02 F submitted with this application are listed in Table 9.10-1 and in Appendix 1 and are summarised in Appendix 2. The seedling emergence and the vegetative vigour studies were conducted with the formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl. Since differences in co-formulants and/or their concentration between both formulations are considered minimal, it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation composition please refer to Part C of this submission.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table ‑: Endpoints and effect values of BAS 743 03 F relevant for the risk assessment for non-target terrestrial plants

| Species | Product | Exposure system | Results | Reference |
| --- | --- | --- | --- | --- |
| *Daucus carota L.* (carrot)  *Lactuca sativa* (lettuce)  *Brassica napus* (oilseed rape)  *Cucumis sativus* (cucumber)  *Glycine max* d (soybean)  *Solanum lycopersicum*d (tomato)  *Allium cepa*m (onion)  *Lolium multiflorum*m (ryegrass)  *Triticum aestivum*m (wheat)  *Zea mays* m (corn) | BAS 743 02 F\* | 21 d  Seedling emergence | ER50 > 3.85 L/ha (emergence, plant height, biomass, phytotoxicity and survival) | New study  2022/2033722 |
| *Daucus carota L.* (carrot)  *Lactuca sativa* (lettuce)  *Brassica napus* (oilseed rape)  *Cucumis sativus* (cucumber)  *Glycine max* d (soybean)  *Solanum lycopersicum*d (tomato)  *Allium cepa*m (onion)  *Lolium multiflorum*m (ryegrass)  *Triticum aestivum*m (wheat)  *Zea mays* m (corn) | BAS 743 02 F\* | 21 d Vegetative vigor | ER50 > 3.85 L/ha  (plant height, biomass, phytotoxicity and survival) | New study  2022/2033723 |

m: monocotyledonous; d: dicotyledonous

\* Tested with the similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCL)

#### Justification for new endpoints

BAS 743 03 F was not the representative formulation during the EU review for approval of the active substance Ametoctradin and Propamocarb. Therefore, the endpoints for the formulation have been taken into account and the risk assessment has been conducted for BAS 743 03 F.

### Risk assessment

#### Tier-1 risk assessment (based screening data)

The evaluation of the risk to non-target plants was performed in accordance with the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). BAS 743 03 F is a fungicide and is therefore not expected to have significant herbicidal activity. Hence, a Tier-1 assessment has been conducted, using the available screening data.

Studies on the effects of BAS 743 03 F exposure on seedling emergence and vegetative vigour of terrestrial higher plants were conducted with a similar formulation BAS 743 02 F containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCL. The results showed that applications up to a rate of 3.85 L BAS 743 02 F/ha (2.5 kg total a.s./ha) caused no reduced seedling emergence and plant fresh weight, and no symptoms of phytotoxicity were observed for any of the ten terrestrial plant species tested.

Since no effects of greater than 50% were observed in the studies at the max application rate, an ER50 > 3.85 L/ha for all tested species based on the aforementioned endpoints was detected.

According to the Terrestrial Guidance Document, the risk to non-target terrestrial plants should be considered acceptable if less than 50% effect on at least six species is seen at the highest nominal application rate (1x). For BAS 743 03 F the highest nominal application rate is 2 L product/ha (1.142 kg total a.s./ha) for all the intended uses. This application rate is much lower (2.2x) than the highest application rate tested on 10 terrestrial plant species, which showed no effects for any of the tested plant species. It can therefore be concluded that the proposed uses of BAS 743 03 F pose no unacceptable risk to non-target terrestrial plants.

#### Tier-2 risk assessment (based on dose-response data)

As the risk assessment based on screening data demonstrates an acceptable risk to non-target terrestrial plants, no further risk assessment is required.

#### Higher-tier risk assessment

As the Tier-1 assessment indicates an acceptable risk to non-target terrestrial plants, no further risk

assessment is required.

#### Risk mitigation measures

No risk mitigation needed.

### Overall conclusions

A Tier-1 risk assessment based on screening data demonstrates an acceptable risk to non-target terrestrial plants for all intended uses of BAS 743 03 F. Particular precautions to reduce the environmental concentrations resulting from BAS 743 03 F applications are not required for the protection of terrestrial non-target plants.

|  |
| --- |
| **Review Comments:**  Based on the risk assessment it can be concluded that the proposed use of BAS 743 03 F poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from BAS 743 03 F applications are not required for the protection of terrestrial non-target plants. |

## Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant.

## Monitoring data (KCP 10.8)

According to the knowledge of the applicant, there are currently no monitoring studies available which assess ecotoxicological effects of BAS 743 03 F or of the containing active substances.

## Classification and Labelling

Plant protection products have to be classified for their acute and chronic environmental hazard according to (EC) No 1272/2008 (CLP). Classification is based primarily on data of the product itself if adequate acute and chronic data is available. When aquatic toxicity data for the formulated product is not available for all three trophic species levels, the summation method is additionally performed, meaning that the content of substances classified with a specific category are added to derive a classification for the product.

For BAS 743 03 F acute data (LC/EC50) are available for all tropic levels from a similar formulation BAS 743 02 F (containing 137.1 g/L Ametoctradin and 515.4 g/L Propamocarb-HCl). Since differences in co-formulants and/or their concentration between both formulations are considered minimal and both formulations are SC (suspension concentrates), it is assumed that bridging from BAS 743 02 F to BAS 743 03 F is acceptable. For details on the formulation compositions please refer to Part C of this submission.

Chronic endpoints (NOEC or EC10) are available only for algae (for BAS 743 02 F), thus chronic classification will be based on the summation method using active substances data. The active substance ametoctradin is classified with H410 (Aquatic Chronic 1), derived from the most sensitive aquatic chronic endpoint. Table 9.13‑1 shows the relevant data for classification purposes.

Table ‑: Ecotoxicology/Environment data relevant for Classification of BAS 743 03 F

| **Substance tested** | **Study Type**  **(duration)** | **Findings** | **Triggered classification and labelling** | **Reference** XXXX DocID |
| --- | --- | --- | --- | --- |
| **Acute (short-term) aquatic hazard** | | | | |
| BAS 743 02 F | *Oncorhynchus mykiss* (96 h) | LC50 > 100 mg/L | No aquatic acute hazard cat. | New study  2022/2033714 |
| BAS 743 02 F | *Daphnia magna* (48 h) | EC50 > 100 mg/L | No aquatic acute hazard cat. | New study  2022/2033712 |
| BAS 743 03 F | *Daphnia magna* (48 h) | EC50 > 100 mg/L | No aquatic acute hazard cat. | New study  2022/2033730 |
| BAS 743 02 F | *Pseudokirchneriella subcapitata* (72 h) | ErC50 > 100 mg/L | No aquatic acute hazard cat. | New study  2022/2033713 |
| **Long-term aquatic hazard** | | | | |
| Ametoctradin (BAS 650 F) 1) | *Americamysis bahia* (28 d) | NOEC = 0.018 mg/L | Aquatic chronic hazard cat. 1 (H410),  M factor = 1 | New study  2013/7000443 |
| Biodegradation | Not readily biodegradable | -- | EFSA Journal 2012;10(11):2921 (2008/1000061) |
| Propamocarb-HCl 2) | *Lepomis macrochirus* (32 d) | NOEC = 6.3 mg/L | No aquatic chronic hazard cat. | EFSA Scientific Report (2006) 78, 1-80 |
| Biodegradation | Not readily biodegradable | -- | EFSA Scientific Report (2006) 78, 1-80 |

Based on the lowest acute aquatic toxicity endpoint obtained with BAS 743 03 F no acute aquatic hazard category is given according to (EC) No 1272/2008 (CLP).

Regarding chronic classification, Propamocarb-HCl (a.s. content of 73% w/w within the product) is not classified and Ametoctradin (a.s. content of 19.5% w/w within the product) is classified as chronic hazard cat. 1. Therefore, Ametoctradin is considered for the summation method in the 1stequation according to CLP (*M x hazard cat. 1*)*,* yielding a value which is below the trigger of 25%. Hence, BAS 743 03 F is not classified as Chronic 1 and thus chronic category 2 is considered using the 2nd equation specified in the CLP Regulation (see Table 9.13‑2). The resulting sum exceeds the trigger of 25 %, hence BAS 743 03 F is classified as chronic aquatic hazard category 2 (H411). Classification of BAS 743 03 F using the summation method is summarized in Table 9.13‑2.

Table ‑: Acute and chronic classification of BAS 743 03 F using the summation method according to (EC) No 1272/2008

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Chronic classification of BAS 743 03 F** | | | | | | | | | |
| Formulation component | | | | | | Result (% Content x M-Factor) |  |  |  |
| Name | Chronic Category | M-Factor | | Content in BAS 743 03 F [%] | |  |  |  |
| Ametoctradin | 1 | 1 | | 19.5 | | 5.7 |  |  |  |
| Propamocarb-HCl | Not classified | -- | | -- | |  |  |  |  |
| 1st equation | SUM *(M x Chronic 1)* | | | | | 19.5 | ≤ 25 % |  | |
| BAS 650 F | 1 | | 1 | | 10 x 19.5 | 195 |  |  | |
| Propamocarb-HCl | Not classified | | -- | | -- | -- |  |  | |
| 2nd equation | SUM *((M x 10 x Chronic 1) + Chronic 2)* | | | | | 195 | ≥ 25 % | **BAS 743 03 F: Aquatic Chronic Hazard Category 2** | |

**Conclusion**

Based on the summation method, the following classification is proposed for BAS 743 03 F: **aquatic chronic hazard category 2 (H411)** according to GHS following Regulation (EC) No 1272/2008.

EUH401: To avoid risks to human health and the environment, comply with the instructions for use.

P391: Collect spillage/liquid.

P501: Dispose of contents/container in accordance with national regulations.

1. Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 10.1.1.1/1 | XXXX | 2022 | BAS 743 02 F: Acute Oral Toxicity Test with Northern Bobwhite (Colinus virginianus)  2022/2033724  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/1 | XXXX | 2023 | BAS 743 02 F - Fish acute - Trout  2022/2033714  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/2 | Wendling, K. | 2023 | BAS 743 02 F: Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test - Semi-static)  2022/2033712  Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.1/3 | Renner, P. | 2023 | Acute toxicity of BAS 743 03 F on Daphnia magna in a 48-hour static test  2022/2033730  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.1/4 | Obert-Rauser, P. | 2023 | Toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindak under Laboratory Conditions  2022/2033713  Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.2/1 | Schwader, A. | 2013 | BAS 650 F - Life-cycle toxicity test with Mysids (Americamysis bahia) following draft OPPTS guideline 850.1350  2013/7000443  Smithers Viscient LLC, Wareham MA, United States of America  yes  Unpublished | No | XXXX |
| KCP 10.3.1.1.1/1 | Poraczki K. | 2023 | Acute toxicity of BAS 743 02 F to the honeybee Apis mellifera L. under laboratory conditions  2022/2033708  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.1.1.1/2 | Poraczki K. | 2023 | Acute toxicity of BAS 743 03 F to the honeybee Apis mellifera L. under laboratory conditions  2022/2033729  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.1.1.1/3 | Amsel, K. | 2023 | Acute toxicity of BAS 743 02 F to the bumblebee Bombus terrestris L. under laboratory conditions  2022/2033711  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.1.1.1/4 | Poraczki K. | 2023 | Amendment No 1 to the report: Acute toxicity of BAS 743 03 F to the honeybee Apis mellifera L. under laboratory conditions  2023/2032255  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| ~~KCP 10.3.1.2/1~~ | ~~Ruhland, S.~~ | ~~2015~~ | ~~Chronic toxicity of BAS 650 00 F to the honeybee (Apis mellifera L.) under laboratory conditions~~  ~~2014/1111114~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | XXXX |
| KCP 10.3.1.2/2 | Ruhland, S. |  | Chronic toxicity of BAS 743 02 F to the honey bee Apis mellifera L. under laboratory conditions  2022/2033709  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| ~~KCP 10.3.1.3/1~~ | ~~Kleebaum, K.~~ | ~~2016~~ | ~~Repeated exposure of BAS 650 00 F to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro)~~  ~~2014/1111115~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | XXXX |
| KCP 10.3.1.3/2 | Schmidt, K. | 2023 | Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 743 02 F under laboratory conditions  2022/2033710  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.2.1/1 | Röhlig,U | 2022 | Effects of BAS 743 02 F on the predatory mite Typhlodromus pyri SCHEUTEN in a laboratory test  2022/2033725  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.2.1/2 | Röhlig,U | 2022 | Effects of BAS 743 02 F on the parasitic wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) in a laboratory test  2022/2033728  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.2.1/3 | Röhlig,U | 2022 | Effects of BAS 743 03 F on the parasitic wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) in a laboratory test  2022/2033732  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.1.1/1 | Friedrich, S. | 2023 | Effects of BAS 743 02 F on the reproduction of the earthworm Eisenia andrei in artificial soil  2022/2033719  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.1.1/2 | Friedrich, S. | 2023 | Effects of BAS 743 03 F on the reproduction of the earthworm Eisenia andrei in artificial soil  2022/2033731  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| ~~KCP 10.4.1.1/3~~ | ~~Friedrich, S.~~ | ~~2007~~ | ~~Sublethal toxicity of BAS 650 00 F to the earthworm Eisenia fetida in artificial soil with 5% peat~~  ~~2007/1037733~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | XXXX |
| KCP 10.4.2.1/1 | Friedrich, S. | 2023 | Effects of BAS 743 02 F on the reproduction of the collembolan Folsomia candida  2022/2033720  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.2.1/2 | Schulz, L. | 2023 | Effects of BAS 743 02 F on the reproduction of the predatory mite Hypoaspis aculeifer  2022/2033721  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| ~~KCP 10.4.2.1/3~~ | ~~Friedrich, S.~~ | ~~2007~~ | ~~Effects of BAS 650 00 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat~~  ~~2007/1037734~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | XXXX |
| ~~KCP 10.4.2.1/4~~ | ~~Schulz, L.~~ | ~~2016~~ | ~~Effects of BAS 650 00 F on the reproduction of the predatory mite Hypoaspis aculeifer~~  ~~2016/1193035~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | XXXX |
| KCP 10.5/1 | Schulz, L. | 2023 | Effects of BAS 743 02 F on the activity of soil microflora (Nitrogen transformation test)  2022/2033717  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.6/2 | Maleck, A. | 2023 | Effect of BAS 743 02 F on seedling emergence and seedling growth of several species of terrestrial plants under greenhouse conditions  2022/2033722  Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.6/3 | Maleck, A. | 2023 | Effect of BAS 743 02 F on vegetative vigour of several species of terrestrial plants under greenhouse conditions  2022/2033723  Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep.  yes  Unpublished | No | XXXX |

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 10.1.1/1 | XXXX | 2007 | BAS 650 F: Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50)  2006/1038389  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.1.1/2 | XXXX | 2007 | BAS 650 F: Acute toxicity in the mallard duck (Anas platyrhynchos) after single oral administration (LD50)  2006/1038390  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.1.1/3 | XXXX | 2008 | BAS 650 F - 1-Generation reproduction study on the bobwhite quail (Colinus virginianus) by administration in the diet  2008/1023023  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.1.1/4 | XXXX | 2008 | Amendment No. 1: BAS 650 F - 1-Generation reproduction study on the bobwhite quail (Colinus virginianus) by administration in the diet  2008/1071959  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.1.1/5 | XXXX | 2009 | Amendment No. 2: BAS 650 F - 1-Generation reproduction study on the bobwhite quail (Colinus virginianus) by administration in the diet  2009/1079840  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.1.1/6 | XXXX | 2007 | BAS 650 F - 1-generation reproduction study on the mallard duck (Anas platyrhynchus) by administration in the diet  2007/1050786  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.1.1/7 | XXXX | 2009 | Amendment No. 1: BAS 650 F - 1-Generation reproduction study on the mallard duck (Anas platyrhynchus) by administration in the diet  2009/1079841  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.1.1/8 | XXXX | 2009 | Amendment No. 2 to the report: BAS 650 F - 1-generation reproduction study on the mallard duck (Anas platyrhynchus) by administration in the diet  2009/1111606  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/1 | XXXX | 2007 | BAS 650 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a flow through system over 96 hours  2007/1004041  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/2 | XXXX | 2007 | BAS 650 00 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours  2007/1057733  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/3 | XXXX | 2007 | BAS 650 F - Acute toxicity on the common carp (Cyprinus carpio) in a flow-through system over 96 hours  2007/1039553  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/4 | XXXX | 2007 | BAS 650 F - Acute toxicity study on the bluegill sunfish (Lepomis macrochirus) in a flow-through-system over 96 hours  2006/1031686  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/5 | Bergtold, M.,  Janson, G. | 2007 | Acute toxicity of BAS 650 F to Daphnia magna STRAUS in a 48 hour static test  2006/1037557  BASF AG, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.1/6 | Hoffmann, F. | 2008 | Effect of BAS 650 F (Reg.No. 4993353) on the growth of the green alga Pseudokirchneriella subcapitata  2008/1034458  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.1/7 | Zok, S. | 2007 | BAS 650 00 F - Determination of the inhibitory effect on the cell multiplication of unicellular green algae Pseudokirchneriella subcapitata  2007/1017586  BASF AG, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.1/8 | Janson, G. | 2008 | Acute toxicity of Reg.No. 5178872 (metabolite of BAS 650 F) to Daphnia magna STRAUS in a 48 hour static test  2008/1034472  BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.1/9 | Gopi, R. | 2008 | Acute toxicity study of Reg.No. 5178870 (metabolite of BAS 650 F) to freshwater fish, Oncorhynchus mykiss  2007/1035788  IIBAT - International Institute of Biotechnology and Toxicology, Padappai, India  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/10 | Gopi, R. | 2008 | Acute immobilisation test with Reg.No. 5178870 (metabolite of BAS 650 F) in Daphnia magna  2007/1035785  IIBAT - International Institute of Biotechnology and Toxicology, Padappai, India  yes  Unpublished | No | XXXX |
| KCP 10.2.1/11 | Ayyappan, A. | 2008 | Effect of Reg.No. 5178870 (metabolite of BAS 650 F) on the growth of green alga, Pseudokirchneriella subcapitata  2007/1035786  IIBAT - International Institute of Biotechnology and Toxicology, Padappai, India  yes  Unpublished | No | XXXX |
| KCP 10.2.1/12 | XXXX | 2008 | Acute toxicity study of Reg.No. 5211623 to freshwater fish, Oncorhynchus mykiss  2007/1035789  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.1/13 | Chittibabu, R. | 2008 | Acute immobilisation test with Reg.No. 5211623 in Daphnia magna  2007/1035784  IIBAT - International Institute of Biotechnology and Toxicology, Padappai, India  yes  Unpublished | No | XXXX |
| KCP 10.2.1/14 | Chandrasehar, G. | 2008 | Effect of Reg.No. 5211623 on the growth of green alga, Pseudokirchneriella subcapitata  2007/1035787  IIBAT - International Institute of Biotechnology and Toxicology, Padappai, India  yes  Unpublished | No | XXXX |
| KCP 10.2.2/1 | XXXX | 2006 | BAS 650 F - Early life-stage test on the fathead minnow (Pimephales promelas) in a flow through system  2006/1024627  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.2/2 | Zok, S. | 2007 | BAS 650 00 F - Determination of the acute effect on the swimming ability of the water flea Daphnia magna STRAUS  2007/1018762  BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.2/3 | Weltje, L. | 2008 | Chronic toxicity of the pyrimidylamine (BAS 650 F) formulation BAS 650 00 F to the non-biting midge Chironomus riparius - A spiked sediment study  2007/1057455  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.2/1 | XXXX | 2006 | BAS 650 F - Early life-stage test on the fathead minnow (Pimephales promelas) in a flow through system  2006/1024627  XXXX  yes  Unpublished | Yes | XXXX |
| KCP 10.2.2/2 | Zok, S. | 2007 | BAS 650 00 F - Determination of the acute effect on the swimming ability of the water flea Daphnia magna STRAUS  2007/1018762  BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.2/3 | Weltje, L. | 2008 | Chronic toxicity of the pyrimidylamine (BAS 650 F) formulation BAS 650 00 F to the non-biting midge Chironomus riparius - A spiked sediment study  2007/1057455  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.2/4 | Rzodeczko, H. | 2008 | M650F03 - Daphnia magna reproduction test  2008/1043909  Institute of Industrial Organic Chemistry, Pszczyna, Poland  yes  Unpublished | No | XXXX |
| KCP 10.2.2/1 | Zok, S. | 2006 | BAS 650 F - Early life-stage test on the fathead minnow (Pimephales promelas) in a flow through system  2006/1024627  BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.  yes  Unpublished | Yes | XXXX |
| KCP 10.2.2/2 | Zok, S. | 2007 | BAS 650 00 F - Determination of the acute effect on the swimming ability of the water flea Daphnia magna STRAUS  2007/1018762  BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.2/3 | Weltje, L. | 2008 | Chronic toxicity of the pyrimidylamine (BAS 650 F) formulation BAS 650 00 F to the non-biting midge Chironomus riparius - A spiked sediment study  2007/1057455  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.2.2/4 | Rzodeczko, H. | 2008 | M650F03 - Daphnia magna reproduction test  2008/1043909  Institute of Industrial Organic Chemistry, Pszczyna, Poland  yes  Unpublished | No | XXXX |
| KCP 10.3.1.1.1/1 | Schmitzer, S. | 2006 | Effects of BAS 650 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory  2006/1012047  Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.1.1.2/1 | Schmitzer, S. | 2006 | Effects of BAS 650 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory  2006/1012047  Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.1.1/1 | Luehrs, U. | 2008 | Effects of Reg.No. 5178870 (metabolite of BAS 650 F, M650F03) on reproduction and growth of earthworms Eisenia fetida in artificial soil  2008/1010610  Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.1.1/2 | Luehrs, U. | 2008 | Effects of Reg.No. 5211623 (metabolite of BAS 650 F, M650F04) on reproduction and growth of earthworms Eisenia fetida in artificial soil  2008/1010609  Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.2.1/1 | Luehrs, U. | 2008 | Effects of Reg.No. 5178870 (metabolite of BAS 650 F, M650F03) on reproduction of the collembola Folsomia candida in artificial soil  2008/1032646  Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.2.1/2 | Luehrs, U. | 2008 | Effects of Reg.No. 5211623 (metabolite of BAS 650 F, M650F04) on reproduction of the collembola Folsomia candida in artificial soil  2008/1032647  Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.2.1/3 | Royer, S. | 2008 | Effects of Reg.No. 5178870 (metabolite of BAS 650 F, M650F03) on reproduction of soil mites Hypoaspis aculeifer in artificial soil  2008/1031478  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.2.1/4 | Royer, S. | 2008 | Effects of Reg.No. 5211623 (metabolite of BAS 650 F, M650F04) on reproduction of soil mites Hypoaspis aculeifer in artificial soil  2008/1031479  BASF SE, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.5/1 | Schulz, L. | 2007 | Effects of BAS 650 00 F on the activity of soil microflora (nitrogen transformation test)  2006/1026123  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.5/2 | Schulz, L. | 2009 | Amendment No. 1: Effects of BAS 650 00 F on the activity of soil microflora (nitrogen transformation test)  2009/1075782  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.5/3 | Schulz, L. | 2008 | Effects of Reg.No. 5178870 (metabolite of BAS 650 F, M650F03) on the activity of soil microflora (nitrogen transformation test)  2008/1033292  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.5/4 | Schulz, L. | 2009 | Amendment No. 1: Effects of Reg.No. 5178870 (metabolite of BAS 650 F, M650F03) on the activity of soil microflora (nitrogen transformation test)  2009/1075790  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.5/5 | Schulz, L. | 2008 | Effects of Reg.No. 5211623 (metabolite of BAS 650 F, M650F04) on the activity of soil microflora (nitrogen transformation test)  2008/1033293  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.5/6 | Schulz, L. | 2009 | Amendment No. 1: Effects of Reg.No. 5211623 (metabolite of BAS 650 F, M650F04) on the activity of soil microflora (nitrogen transformation test)  2009/1075792  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.5/7 | Schulz, L. | 2009 | Amendment No. 1: Effects of Reg.No. 5211623 (metabolite of BAS 650 F, M650F04) on the activity of soil microflora (nitrogen transformation test)  2009/1075786  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 10.3.1.2/1 | Ruhland, S. | 2015 | Chronic toxicity of BAS 650 00 F to the honeybee (Apis mellifera L.) under laboratory conditions  2014/1111114  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.3.1.3/1 | Kleebaum, K. | 2016 | Repeated exposure of BAS 650 00 F to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro)  2014/1111115  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.1.1/3 | Friedrich, S. | 2007 | Sublethal toxicity of BAS 650 00 F to the earthworm Eisenia fetida in artificial soil with 5% peat  2007/1037733  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.2.1/3 | Friedrich, S. | 2007 | Effects of BAS 650 00 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat  2007/1037734  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |
| KCP 10.4.2.1/4 | Schulz, L. | 2016 | Effects of BAS 650 00 F on the reproduction of the predatory mite Hypoaspis aculeifer  2016/1193035  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | XXXX |

List of data relied on not submitted by the applicant but necessary for evaluation

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP XX | Author | YYYY | Title  Company Report N  Source  GLP/non GLP/GEP/non GEP  Published/Unpublished | Y/N | Owner |
|  |  |  |  |  |  |

1. Detailed evaluation of the new studies
   1. KCP 10.1 Effects on birds and other terrestrial vertebrates
      1. KCP 10.1.1 Effects on birds
         1. KCP 10.1.1.1 Acute oral toxicity
            1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guideline 223 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Reference: | CP 10.1.1.1/1 |
| Report | BAS 743 02 F: Acute Oral Toxicity Test with Northern Bobwhite (*Colinus virginianus*)  XXXX. 2022  XXXX Study ID: 933752\_15  XXXX Doc ID: 2022/2033724  Authority registration No |
| Guideline(s): | OECD 223 (2016) |
| Deviations: | No |
| GLP: | yes | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The acute oral toxicity of BAS 743 02 F to the Northern bobwhite quail (*Colinus virginianus*) was determined in the laboratory over 14 days as a limit test with a nominal dose of 2000 mg product/kg body weight (bw), administered as oral gavage. An untreated control group was tested in parallel. Each treatment comprised of one group containing ten Northern bobwhite (five males and five females).

Birds were observed on multiple occasions during day 0, and once daily for the remainder of the study. Observations were performed for mortality, signs of toxicity, abnormal behaviour, and signs of regurgitation. Body weights were measured individually at test initiation and on days 3, 7 and 14. Feed consumption was determined for days 0-3, 3-7 and 7-14.

No mortalities occurred during the study. No abnormal behaviour was observed for the control group. All birds in the 2000 mg product/kg bw group exhibited reduced faecal urate production or production of only or primarily urates, but all birds returned to normal faecal urate production by day 2 post-dosing. Body weight change and feed consumption were within normal limits for both the control and the 2000 mg product/kg bw treatment group throughout the experimental phase of the study. The LD50 was estimated to be > 2000 mg product/kg bw, i.e. greater than the nominal dose tested.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |  |
| --- | --- | --- |
| **1.** | **Test material:** | BAS 743 02 F |
|  | **Description:** | Suspension concentrate (SC) |
|  | **Active Substances:** | Propamocarb : 432.0 g/L nominal (analysed: 431.0 g/L)  BAS 650 F (Ametoctradin): 137.14 g/L nominal (analysed: 137.7 g/L) |
|  | **Lot/Batch:** | FRE-002224 |
|  |  |  |
| **2.** | **Control:** | Untreated distilled water |

**B. STUDY DESIGN AND METHODS**

|  |  |  |
| --- | --- | --- |
| **1.** | **Test organism:** | Northern bobwhite quail (*Colinus virginianus*) |
|  | **Age:** | Adults, 47 weeks and 4 days old at test initiation |
|  | **Body weight:** | 195.0 - 217.3 g |
|  | **Source:** | Woodland Acres Hatchery and Game Farm, 925 190th Ave, Fairmont, Minnesota 56031, United States; Identified as Smithers Colony No. 22-A-02 |
|  | **Acclimation:** | 2 weeks |
|  |  |  |
| **2.** | **Diet:** | Purina Game Bird Flight Conditioner provided *ad libitum*. Food was withheld for approximately 14 hours and 22 minutes during overnight dark hours prior to dosing. |
|  |  |  |
| **3.** | **Housing:** | 51 × 25.5 × 21 to 25 cm test cages constructed of epoxy-coated wire mesh |
|  |  |  |
| **4.** | **Environmental conditions:** | |
|  | **Temperature:** | Acclimation: 22.5 - 27.0 °C; exposure phase: 20.6 to 26.9 °C; |
|  | **Relative humidity:** | Acclimation: 63.5 to 82.2%; exposure phase: 41.0 to 67.0% |
|  | **Photoperiod:** | 8 hours light : 16 hours dark (mean lux 113), with 15-minute morning and evening transition periods. |

**5. Animal assignment and treatment:**

The acute oral toxicity of BAS 743 02 F to the Northern bobwhite quail (*Colinus virginianus*) was determined in the laboratory over 14 days as a limit test with a nominal dose of 2000 mg product/kg bw, administered as oral gavage. An untreated control group was tested in parallel. Each treatment comprised of one group containing ten northern bobwhite (five males and five females, randomly assigned). After dosing, food and water were provided *ad libitum*.

**6. Dose preparation:**

The liquid test substance was delivered neat, using a 1-mL syringe with 0.01-mL graduations and a 16-gauge × 3-inch stainless steel, ball-tipped animal feeding needle to draw and deliver the appropriate volume of test substance to each bird. Birds in the control group were dosed with distilled water using the density of the test substance to achieve a similar volume for both groups. Doses were delivered into the distal end of each bird’s esophagus, just superior to the proventriculus.

**7. Measurements and observations:**

Following dosing, multiple observations were performed on day 0 of the test, with particular attention being paid to signs of regurgitation. From test initiation until termination, observations were performed daily. A record was maintained of all mortality, signs of toxicity and abnormal behaviour. Body weights were measured individually at the initiation of the test and on days 3, 7 and 14 of the test. Average feed consumption was determined by pen for each dosage group and the control group for days 0-3, 3-7 and 7-14.

**8. Statistics:**

There were no mortalities during the study, therefore it was not possible to perform the calculation of an LD50.  The LD50 value was determined to be greater than the highest dose tested. No statistical analyses were applied to separate mean responses among treatment groups for the endpoints of food consumption and body weight.

**II. RESULTS AND DISCUSSION**

**A. MORTALITY AND BEHAVIOUR**

There were no mortalities in the control or treatment group. No abnormal behaviour was observed for the control group. All birds in the 2000 mg product/kg bw group exhibited reduced faecal urate production or production of only or primarily urates, but all birds returned to normal faecal urate production by day 2 post-dosing. Body weight change and feed consumption was within normal limits for both the control and 2000 mg product/kg bw treatment groups throughout the experimental phase of the study. Therefore, the LD50 was estimated to be greater than 2000 mg product/kg bw.

**B. BODY WEIGHT AND FEED CONSUMPTION**

There were no treatment related effects on body weight or feed consumption among the birds in the test item treatments compared to the control. Mean body weight data are presented in Table A 1and mean feed consumption data are presented in Table A 2.

Table A 1: Mean body weights of Northern bobwhites over 14 days

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment group  (mg product/kg bw)** | **Mean body weights [g] (SD)** | | | | |
| **Day 1** | **Day 3** | **Day 7** | **Day 14** | **Total change**  **Day -1 to Day 14** |
| Control | 204.1 (6.8) | 209.7 (6.7) | 205.6 (7.6) | 208.8 (7.1) | 4.7 (1.2) |
| 2000 | 211.5 (6.1) | 215.7 (6.3) | 214.1 (6.1) | 215.7 (5.7) | 4.1 (2.4) |

SD: Standard deviation

Table A 2: Estimated mean feed consumption of Northern bobwhites over 14 days

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment group  (mg product/kg bw)** | **Average Feed Consumption/Bird/Day [g] (SD)** | | |
| **Days 1 – 3** | **Days 3 – 7** | **Days 7 - 14** |
| Control | 14.6  (1.8) | 12.8  (1.0) | 13.3  (1.1) |
| 2000 | 12.4  (3.8) | 15.5  (1.5) | 15.1  (1.6) |

SD: Standard deviation

**C. VALIDITY CRITERIA**

The study was performed according to OECD 223 (2016). Therefore, the study has been compared to the validity criteria associated with this guideline. The study fulfilled the validity criteria outlined in the guideline (OECD 223, 2016), as detailed below:

* Less than 10% of control birds died during the test (actual value: 0%).
* No additional control birds should be added during the course of the study (actual value: 0).

**III. CONCLUSION**

The acute oral toxicity of BAS 743 02 F to the Northern bobwhite quail (*Colinus virginianus*) was determined in the laboratory over 14 days as a limit test with a nominal dose of 2000 mg product/kg bw, administered as oral gavage. No mortalities occurred during the study and there were no treatment-related effects on body weight or feed consumption. The LD50 was estimated to be > 2000 mg product/kg bw, i.e. greater than the nominal dose tested.

* + - 1. KCP 10.1.1.2 Higher tier data on birds

No further studies were conducted.

* + 1. KCP 10.1.2 Effects on terrestrial vertebrates other than birds
       1. KCP 10.1.2.1 Acute oral toxicity to mammals

Please refer to Appendix 2 of Section 6 (Toxicology), Point KCP 7.1.1.

* + - 1. KCP 10.1.2.2 Higher tier data on mammals

No further studies were conducted.

* + 1. KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

No further studies were conducted.

* 1. KCP 10.2 Effects on aquatic organisms
     1. KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes
        1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guideline 203 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Reference: | CP 10.2.1/1 |
| Report | BAS 743 02 F: Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test – Semi-Static)  XXXX, 2023a  XXXX Study ID: 933752-6  XXXX Doc ID: 2022/2033714 |
| Guideline(s): | Biological part: OECD 203 (2019)  Analytical part: SANTE/2020/12830, Rev.1, 24. |
| Deviations: | No |
| GLP: | Yes | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The acute toxicity of the BAS 743 02 F to rainbow trout (*Oncorhynchus mykiss*) was investigated in a 96-hour semi-static test with the nominal concentrations 0 (control), 4.27, 9.39, 20.7, 45.5 and 100 mg product/L. Each treatment group consist on one replicate containing seven fish. Observations for mortality and abnormal effects were made at test initiation and after 2-3, 5-6 hours and once every approx. 24 hours.

The 96-hour LC50 of BAS 743 02 F to rainbow trout (*Oncorhynchus mykiss*) was above the highest concentration tested of 100 mg product/L based on nominal concentrations. No sublethal effects were observed in the control and test item concentrations up to and including 9:39 mg product/L after 96 hours. Sublethal effects observed in test item concentrations from 20.7 up to and including 100.0 mg product/L were loss of equilibrium and abnormal swimming behaviour.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| **Test item:** | BAS 743 02 F |
| **Description:** | White liquid, soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Ametoctradin: nominal 137.14 g/L; analysed 137.7 g/L,  Propamocarb: nominal 432.0 g/L; analysed 431.0 g/L |
| **Density:** | 1.080 g/cm3 |
| **Storage conditions:** | 5 °C – 30 °C , dark and dry |
| **Stability (expiry date):** | 31.01.2024 |
| **Control:** | Untreated test medium |
| **Reference item:** | Not relevant |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. **Test organism:** | Rainbow trout(*Oncorhynchus mykiss*) Walbaum |
| **Size:** | Fish were selected with a length between 3 and 6 cm at test start |
| **Source:** | Forellenzucht (trout farm) Peter Störk, D-88348 Bad Saulgau, Germany |
| **Acclimatisation:** | All fish were held in the laboratory for at least 12 days before the start of the test. The fish were held under testing conditions in 300 L containers with continuous renewal of water (5 - 10 % per day) and permanent aeration of water for the entire pre-experimental period. |
| **Diet:** | During acclimatisation period: daily with granular rearing food (‘INICIO Plus G’, 2.0 mm, BioMar, DK) *ad libitum* until 24 hours before the test was started; Fish were not fed during the exposure period |
| 1. **Test units:** | 18 L glass aquaria with 15 L test medium |
| 1. **Environmental conditions** |  |
| **Temperature:** | 12.3 – 13.2°C |
| **pH:** | 7.64 – 7.82 |
| **Dissolved O2 concentration:** | 83.6 ± 9.3 % of air saturation (71 – 96 %) |
| **Hardness:** | 11°dH corresponding to 196 mg CaCO3/L |
| **Photoperiod:** | 16 h light : 8 h dark; 30 min dawn/dusk period |
| **Light intensity:** | 832 Lux at test start |
| **Aeration of the test water:** | None |
| **Loading:** | 0.54 g fish/L (in control group) |

1. **Treatment:**

The study was conducted as a semi-static dose-response test with five test item concentrations, i.e. 4.27, 9.39, 20.7, 45.5 and 100 mg product/L (nominal), and one control with untreated test medium. Non-GLP pre-experiments were performed to determine a suitable concentration range. Each test concentration and control contained seven fish (i.e. one replicate per treatment group with seven fish per replicate). The duration of the test was 96 hours. The test was performed without a reference item.

1. **Dose preparation:**

A stock solution of 100 mg test item/L was prepared by dissolving 3000 mg test item into 1000 mL test water by intense shaking. Adequate volumes of this stock solution were diluted with test water to prepare the test media of the desired test concentrations. All stock solutions were homogenized by shaking and afterwards they appeared to be turbid. The test concentrations were prepared by dissolving 500 mL of the stock solutions in 15 L test medium in a glass aquarium. The test media were prepared just before introduction of the test fish (= start of the test).

1. **Measurements and observations:**

Biological observations:

The test fish were observed for sublethal effects and mortality at test start and after approximately 2-3 hours, 5-6 hours, 24 hours, 48 hours, 72 hours and 96 hours. Fish were considered dead if there was no visible movement (e.g. gill movement), and if touching of the caudal peduncle produced no reaction. Records were made on visible abnormalities such as: difficulties with maintenance of equilibrium, swimming behaviour, respiratory function, pigmentation and all other observed effects. Dead fish were removed. At termination of the test, all remaining fish were euthanized. Fish of the control group were then weighed and measured.

Physicochemical measurements:

Measurements of temperature, pH and oxygen saturation were performed in 24-hour intervals in all test concentrations and control. Water hardness of the untreated control was determined at the beginning of the test. Light intensity was measured at test start. Assessment of test solution appearance was done during each biological assessment.

Analytical verification:

Analytical samples were taken at 0 hours (fresh), 24 hours, 48 hours, 72 hours (fresh and aged) and 96 hours (aged) from control and all test item concentrations. For each sampling, also two retain samples were taken. All samples were stored deep frozen until they were transferred to the analytical laboratory.

The analytical verification of test item concentrations in fish test medium was done by analysing the content of ametoctradin and propamocarb in the samples collected during the test. The content of the active substances in the test samples was determined by analysing with HPLC-MS/MS.

1. **Statistical analysis:**

No statistical analysis was performed. The LC50 could not be quantified due to the absence of toxicity of the test item. The NOEC and the LOEC were determined directly from the raw data.

1. **Description of the analytical procedures**

The analytical verification of test item concentrations in fish test medium was done by analysing the content of ametocradin and propamocarb in the samples during the test. XXXX analytical method APL0500/02 was modified for the analysis of ametoctradin and propamocarb.

Sample analysis was performed by dilution of the sample with 50 µL formic acid and the equal amount of acetonitrile. Subsequently, the samples were diluted with acetonitrile/test medium + 0.5 % formic acid (1:1, v/v) to be within the range of the calibration curve. Final determination was accomplished by LC-MS/MS. The method has a limit of quantification (LOQ) of 0.0547 mg ametoctradin/L and 0.170 mg propamocarb/L. The limit of detection (LOD) is of 0.0160 mg ametoctradin/L and 0.0480 mg propamocarb/L.

Blank control samples (blanks of the carrier without test item) were analysed within this study. No significant interference from the blank carrier within the test item could be observed. The storage stabilities of ametoctradin and propamocarb were investigated by fortification of at least six untreated test medium with the test item. Three of the samples were analysed on the day of fortification (day 0). The other fortified samples were stored frozen (≤ 18°C). After 37 days of storage, three samples were analysed. This time period covers the longest storage period of samples of 35 days in test medium. The results show that both analytes were stable under deep-frozen conditions (≤ 18 °C) in test medium for 37 days.

Results of the validation and procedural recovery experiments obtained during analysis showed that the recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for analyte BAS 743 02 F, with relative standard deviations RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of BAS 743 02 F.

The validity criteria (specificity, linearity, accuracy and precision) for the analytical method have been met according to SANTE/2020/12830 Rev. 1 (2021).

Table A 3: Procedural recoveries for ametoctradin and propamocarb applied as BAS 743 02 F in test water

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Analyte** | **Fortification Level [mg/L]** | **n** | **Recoveries [%]** | **Mean Recovery [%]** | **RSD [%]** |
| Ametoctradin | Control | 2 | <LOD | - | - |
| 0.427 | 16 | 84.0, 88.0, 95.0, 93.0, 91.0, 87.0, 85.0, 85.0, 86.0, 89.0, 89.0, 88.0, 88.0, 96.0, 99.0, 98.0 | 90.0 | 5.0 |
| 130 | 14 | 101, 108, 105, 105, 104, 103, 104, 104, 100, 99.0, 98.0, 105, 105, 107 | 103 | 3.0 |
| Overall | 30 | - | 96.0 | 8.0 |
| Propamocarb | Control | 2 | <LOD | - | - |
| 0.427 | 16 | 99.0, 100, 104, 99.0, 100, 111, 111, 112, 101, 101, 102, 102, 101, 106, 108, 107 | 104 | 4.0 |
| 130 | 14 | 99.0, 106, 102, 101, 103, 102, 103, 99.0, 99.0, 98.0, 98.0, 103, 104, 103 | 101 | 2.0 |
| Overall | 30 | - | 103 | 4.0 |

1. **Results and Discussion**
2. **ANALYTICAL RESULTS**

The contents of analyte in the test concentrations were determined to confirm the correct application of the test item. The measured content of ametoctradin was between 114 % and 130 % of nominal at test start and between 92 % and 105 % of nominal at the end in the respective test concentrations. The measured content of propamocarb was between 109 % and 122 % of nominal at test start and between 95 % and 99 % of nominal at the end in the respective test concentrations. Since the measured concentrations of ametoctradin in aged test solutions were not between 80 and 120 % of nominal concentration, the biological endpoints were evaluated using the nominal concentrations and the actual concentrations of the test item (based on the geometric mean of fresh and aged measured concentrations). The analytical results are presented in the following tables.

Table A 4: Measured concentrations of ametoctradin in test water

| **Test item [mg product/L]** | **Sampling Time** | **Nominal Concentration [mg a.s./L]** | **Measured Concentration [mg a.s./L]** | **Recovery [%]** |
| --- | --- | --- | --- | --- |
| Control | 0 h fresh | 0.0 | - | <LOD |
| 24 h fresh | 0.0 | - | <LOD |
| 24 h aged | 0.0 | - | <LOD |
| 48 h fresh | 0.0 | - | <LOD |
| 48 h aged | 0.0 | - | <LOD |
| 72 h fresh | 0.0 | - | <LOD |
| 72 h aged | 0.0 | - | <LOD |
| 96 h aged | 0.0 | - | <LOD |
| 4.27 | 0 h fresh | 0.547 | 0.710 | 130 |
| 24 h fresh | 0.547 | 0.630 | 115 |
| 24 h aged | 0.547 | 0.616 | 113 |
| 48 h fresh | 0.547 | 0.688 | 126 |
| 48 h aged | 0.547 | 0.588 | 107 |
| 72 h fresh | 0.547 | 0.540 | 99.0 |
| 72 h aged | 0.547 | 0.640 | 117 |
| 96 h aged | 0.547 | 0.504 | 92.0 |
| 9.39 | 0 h fresh | 1.20 | 1.40 | 117 |
| 24 h fresh | 1.20 | 1.34 | 112 |
| 24 h aged | 1.20 | 1.25 | 104 |
| 48 h fresh | 1.20 | 1.37 | 114 |
| 48 h aged | 1.20 | 1.19 | 99.0 |
| 72 h fresh | 1.20 | 1.26 | 105 |
| 72 h aged | 1.20 | 1.30 | 108 |
| 96 h aged | 1.20 | 1.12 | 93.0 |
| 20.7 | 0 h fresh | 2.65 | 3.01 | 114 |
| 24 h fresh | 2.65 | 2.73 | 103 |
| 24 h aged | 2.65 | 2.51 | 95.0 |
| 48 h fresh | 2.65 | 3.08 | 116 |
| 48 h aged | 2.65 | 2.72 | 103 |
| 72 h fresh | 2.65 | 2.75 | 104 |
| 72 h aged | 2.65 | 2.79 | 105 |
| 96 h aged | 2.65 | 2.61 | 98.0 |
| 45.5 | 0 h fresh | 5.82 | 6.90 | 119 |
| 24 h fresh | 5.82 | 6.86 | 118 |
| 24 h aged | 5.82 | 5.88 | 101 |
| 48 h fresh | 5.82 | 6.14 | 105 |
| 48 h aged | 5.82 | 6.04 | 104 |
| 72 h fresh | 5.82 | 6.10 | 105 |
| 72 h aged | 5.82 | 5.70 | 98.0 |
| 96 h aged | 5.82 | 5.78 | 99.0 |
| 100 | 0 h fresh | 12.8 | 14.6 | 114 |
| 24 h fresh | 12.8 | 13.5 | 105 |
| 24 h aged | 12.8 | 13.5 | 105 |
| 48 h fresh | 12.8 | 14.3 | 112 |
| 48 h aged | 12.8 | 12.8 | 100 |
| 72 h fresh | 12.8 | 14.2 | 111 |
| 72 h aged | 12.8 | 13.6 | 106 |
| 96 h aged | 12.8 | 13.5 | 105 |

LOQ = 0.0547 mg Ametoctradin/L, LOD = 0.00160 mg Ametoctradin/L

Table A 5: Measured concentrations of Propamocarb in test water

| **Test item [mg product/L]** | **Sampling Time** | **Nominal Concentration [mg a.s./L]** | **Measured Concentration [mg a.s./L]** | **Recovery [%]** |
| --- | --- | --- | --- | --- |
| Control | 0 h fresh | 0.0 | - | <LOD |
| 24 h fresh | 0.0 | - | <LOD |
| 24 h aged | 0.0 | - | <LOD |
| 48 h fresh | 0.0 | - | <LOD |
| 48 h aged | 0.0 | - | <LOD |
| 72 h fresh | 0.0 | - | <LOD |
| 72 h aged | 0.0 | - | <LOD |
| 96 h aged | 0.0 | - | <LOD |
| 4.27 | 0 h fresh | 1.70 | 2.08 | 122 |
| 24 h fresh | 1.70 | 1.89 | 111 |
| 24 h aged | 1.70 | 2.00 | 118 |
| 48 h fresh | 1.70 | 1.97 | 116 |
| 48 h aged | 1.70 | 1.97 | 116 |
| 72 h fresh | 1.70 | 1.57 | 92.0 |
| 72 h aged | 1.70 | 2.06 | 121 |
| 96 h aged | 1.70 | 1.61 | 95.0 |
| 9.39 | 0 h fresh | 3.75 | 4.48 | 119 |
| 24 h fresh | 3.75 | 4.04 | 108 |
| 24 h aged | 3.75 | 4.20 | 112 |
| 48 h fresh | 3.75 | 4.16 | 111 |
| 48 h aged | 3.75 | 4.16 | 111 |
| 72 h fresh | 3.75 | 3.70 | 99.0 |
| 72 h aged | 3.75 | 4.28 | 114 |
| 96 h aged | 3.75 | 3.64 | 97.0 |
| 20.7 | 0 h fresh | 8.26 | 9.40 | 114 |
| 24 h fresh | 8.26 | 8.15 | 99.0 |
| 24 h aged | 8.26 | 8.06 | 98.0 |
| 48 h fresh | 8.26 | 8.87 | 107 |
| 48 h aged | 8.26 | 9.22 | 112 |
| 72 h fresh | 8.26 | 8.11 | 98.0 |
| 72 h aged | 8.26 | 8.96 | 108 |
| 96 h aged | 8.26 | 8.05 | 97.0 |
| 45.5 | 0 h fresh | 18.2 | 20.2 | 111 |
| 24 h fresh | 18.2 | 20.8 | 114 |
| 24 h aged | 18.2 | 18.6 | 102 |
| 48 h fresh | 18.2 | 18.3 | 101 |
| 48 h aged | 18.2 | 19.8 | 109 |
| 72 h fresh | 18.2 | 17.8 | 98.0 |
| 72 h aged | 18.2 | 18.7 | 103 |
| 96 h aged | 18.2 | 18.0 | 99.0 |
| 100 | 0 h fresh | 39.9 | 43.4 | 109 |
| 24 h fresh | 39.9 | 40.7 | 102 |
| 24 h aged | 39.9 | 40.1 | 101 |
| 48 h fresh | 39.9 | 41.1 | 103 |
| 48 h aged | 39.9 | 39.7 | 99.0 |
| 72 h fresh | 39.9 | 41.6 | 104 |
| 72 h aged | 39.9 | 41.4 | 104 |
| 96 h aged | 39.9 | 39.2 | 98.0 |

LOQ = 0.170 mg Propamocarb/L, LOD = 0.0480 mg Propamocarb/L

1. **biological effects**

In the control and at the test item concentrations up to 100.0 mg product/L, all fish survived until the end of the experiment. No sublethal effects were observed in the control and test item concentrations up to and including 9.39 mg product/L. Sublethal effects observed in test item concentrations from 20.7 up to and including 100 mg product/L were loss of equilibrium and abnormal swimming behaviour. Biological results are presented in the following table.

Table A 6: Mortality of rainbow trout (*Oncorhynchus mykiss*) exposed to BAS 743 02 F for 96 hours

| **Nominal concentration [mg product/L]** | **Cumulative mortality [%]** | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| **0 h** | **2 h** | **24 h** | **48 h** | **72 h** | **96 h** |
| Control | 0 | 0 | 0 | 0 | 0 | 0 |
| 4.27 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9.39 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 | 0 | 0 | 0 |

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 7: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 203 (2019)** | **Obtained in this study** |
| In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure; | 0% |
| The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test. | ≥ 71 % |
| Analytical measurement of test concentrations is compulsory | Analysis of each test conc. at test initiation (0-hour) and after 96-hour |

1. **DEFICIENCIES**

There were no deviations with impact on quality and integrity of the study.

1. **Conclusion**

The 96-hour LC50 of BAS 743 02 F to rainbow trout (*Oncorhynchus mykiss*) was above the highest concentration tested of 100 mg product/L, based on nominal concentration. No sublethal effects were observed in the control and test item concentrations up to and including 9:39 mg product/L after 96 hours. Sublethal effects observed in test item concentrations from 20.7 up to and including 100.0 mg product/L were loss of equilibrium and abnormal swimming behaviour.

* + - 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.2.1/02 |
| Report | BAS 743 02 F: Toxicity to the Water Flea Daphnia magna Straus under Laboratory Conditions (Acute Immobilisation Test – Static)  Wendling, K., 2023b  XXXX Study ID: 933752\_4  XXXX Doc ID: 2022/2033712 |
| Guideline(s): | Biological part: OECD 202 (2004)  Analytical part: SANCO/3029/99 rev.4 11/07/00 |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The acute toxicity of the test item BAS 743 02 F to *Daphnia magna* was investigated in a 48-hour semi-static test with the nominal concentrations 0 (control), 4.27, 9.39, 20.7, 45.5 and 100.0 mg product/L. Four replicates containing five Daphnia each were used for all treatment groups.

The 48-hour EC50 of BAS 743 02 F to *Daphnia magna* was above the highest concentration tested of 100 mg product/L based on nominal concentrations. The NOEC was determined to be ≥ 100.0 mg product/L BAS 743 02 F.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. Test item: | BAS 743 02 F |
| Description: | Liquid, soluble concentrate (SC) |
| Lot/Batch: | FRE-002224 |
| Active substance content: | Propamocarb: nominal 432.0 g/L; analysed 431.0 g/L,  Ametoctradin: 137.14 g/L; analysed 137.7 g/L |
| Density: | 1.080 g/cm3 |
| Storage conditions: | 5 °C – 30 °C , dark and dry |
| Stability (expiry date): | 31.01.2024 |
| 1. Control: | Untreated test medium |
| 1. Reference item: | Potassium dichromate tested in a separate study to verify test system sensitivity. |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. Test organism: | *Daphnia magna* (Straus), Clone V |
| Age | < 24 hours |
| Source: | In-house laboratory culture, originally purchased from Federal Environment Agency in Berlin, Germany. |
| Culture medium: | Elendt M4 medium |
| Acclimatisation: | Not necessary since the test was performed in the same medium and under similar temperature and light conditions as in culturing  meium and under similar temperature and light conditions as in culturing |
| Diet: | No feeding during the test, before exposure fed with single cell green algae (*Desmodesmus subspicatus*) three times per week |
| 1. Test units: | Glass vessel (100 mL), filled up with ≥ 50 mL test solution and covered with a glass plate |
| 1. Environmental conditions |  |
| Temperature: | 19.1 – 20.1 °C |
| pH: | 7.68 – 7.94 |
| Dissolved O2 concentration: | ≥ 7.91 mg/L (8.70 ± 0.45 mg/L) |
| Hardness: | 14°dH (250 mg/L CaCO3) |
| Photoperiod: | 16 h light : 8 h dark |
| Light intensity: | 929 lux at test start |
| Aeration of the test water: | No aeration of test vessels |

1. **Treatment:**

The study was conducted as a semi-static dose-response test with five test item nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100.0 mg product/L, and one control with untreated test medium, following a non-GLP range-finding study. The test media were prepared just before introduction of the daphnids (= start of the test). Four replicates containing five Daphnia each were used for all treatment groups and the control. The duration of the test was 48 hours.

1. **Dose preparation:**

The necessary amount of test item for preparing the highest test solution (100 mg product/L) was weighed on a weighing scoop and transferred to a volumetric flask. Test medium was added up to the bench mark and the solution was homogenised by shaking. Lower test solutions were prepared by dilution of the stock solution with test medium. Test solutions of 20.7 – 100 mg product/L appeared turbid and test solutions 4.27 and 9.39 mg product/L appeared clear and transparent. Approximately 50 mL of the prepared solutions were transferred to each test vessel.

1. **Measurements and observations:**

Biological observations:

The mobility of the daphnids was determined by visual observation after 24 and 48 hours. Those animals not able to swim within 15 seconds after gentle agitation of the test vessels were considered to be immobile. If present, behavioural changes and abnormal appearance of daphnids were recorded at 24 and 48 hours after starting the test. Daily observation of test solution appearance was performed.

Physicochemical measurements:

The test temperature and the pH as well as the oxygen concentration of the test solutions were measured at all treatments at test start (fresh), after 24 hours (fresh and aged) and at test termination from aged test solutions in one separate replicate per treatment without test organisms.

Analytical verification:

Analytical samples were taken from all test item concentrations and control at test start from fresh solutions, after 24 hours (fresh and aged) and after 48 hours from aged solutions. For each sampling also two retain samples were taken. For all test medium samples a volume of 10 mL test solution was taken in 20 mL glass vials. All samples were stored deep frozen (≤ - 18 °C) until they were transferred to the analytical laboratory.

Concentrations of the test item BAS 743 02 F were determined via its active substacnes propamocarb and BAS 650 F (ametoctradin) in test item solutions. The determination was conducted by using high performance liquid chromatography (HPLC) with mass-spectrometric (MS-MS) detection.

1. **Statistical analysis:**

ECx could not be determined due to the absence of toxicity of the test item. LOEC and NOEC were determined directly from the raw data. No statistical analysis was performed.

1. **Description of the analytical procedures**

Concentrations of the test item BAS 743 02 F were determined via its active substances propamocarb and ametoctradin in test item solutions using XXXX modified analytical method APL0500/02. The determination was performed using HPLC-MS/MS.

Sample analysis was performed by dilution of the sample with 50 µL formic acid and the equal amount of acetonitrile. Subsequently, the samples were diluted with acetonitrile/test medium + 0.5 % formic acid (1:1, v/v) to be within the range of the calibration curve. The method has a limit of quantification (LOQ) of 0.0547 mg ametoctradin/L and 0.170 mg propamocarb/L. The limit of detection (LOD) is 0.0156 mg ametoctradin/L and 0.0468 mg propamocarb/L.

The storage stabilities of ametoctradin and propamocarb were investigated by fortification of at least six untreated test medium with the test item. Three of the samples were analysed on the day of fortification (day 0). The other fortified samples were stored frozen (≤ 18°C). After 12 days of storage, three samples were analysed. This time period covers the longest storage period of samples of 3 days for propamocarb and 6 days for ametoctradin for test medium. Blank control samples (blanks of the carrier without test item) were analysed within this study. No significant interference from the blank carrier within the test item could be observed. Matrix-matched calibration standards were used for procedural recovery experiments and residue analysis.

Results of the validation and procedural recovery experiments obtained during analysis showed that the recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for analyte BAS 743 02 F, with relative standard deviations RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of BAS 743 02 F.

The validity criteria (specificity, linearity, accuracy and precision) for the analytical method have been met according to SANTE/2020/12830, Rev.1.

Table A 8: Procedural recoveries for ametoctradin and propamocarb applied as BAS 743 02 F in test water

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample Matrix** | **Fortification Level [mg/L]** | **n** | **Recoveries [%]** | **Mean Recovery [%]** | **RSD [%]** |
| Ametoctradin | Control | 2 | <LOD | - | - |
| 0.427 | 5 | 99.0, 102, 102, 106, 102 | 102 | 2.0 |
| 130 | 5 | 105, 105, 105, 101, 103 | 104 | 2.0 |
| Overall | 17 | - | 103 | 2.0 |
| Propamocarb | Control | 2 | <LOD | - | - |
| 0.427 | 5 | 105, 107, 108, 110, 107 | 107 | 2 |
| 130 | 5 | 105, 107, 105, 102, 103 | 104 | 2 |
| Overall | 17 | - | 106 | 2 |

1. **Results and Discussion**
2. **ANALYTICAL RESULTS**

The content of both active substances propamocarb and ametoctradin in test solutions was analytically determined to confirm the correct application of the test item BAS 743 02 F. The measured content of ametoctradin was between 99 % and 104 % of nominal at test start and between 38 % and 53 % of nominal at the end of exposure in the respective test concentrations. The measured content of propamocarb was between 100 % and 103 % of nominal at test start and between 100 % and 108 % of nominal at the end of exposure in the respective test concentrations. Since the measured concentrations of BAS 743 02 F in aged test solutions were not between 80 and 120 % of nominal concentration, the biological endpoints were evaluated using the nominal concentrations and the mean measured concentrations of the test item (based on the geometric mean of fresh and aged measured concentrations of BAS 740 02 F). The analytical results are presented in the following tables.

Table A 9: Measured concentrations of propamocarb in test water

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test item [mg product/L]** | **Timepoint** | **Nominal Concentration [mg a.s./L]** | **Measured Concentration**  **[mg a.s./L]** | **Recovery [%]** |
| Control | 0 h fresh | 0.0 | <LOD | - |
| 24 h aged | 0.0 | <LOD | - |
| 24 h fresh | 0.0 | <LOD | - |
| 48 h aged | 0.0 | <LOD | - |
| 4.27 | 0 h fresh | 1.70 | 1.75 | 103 |
| 24 h aged | 1.70 | 1.71 | 101 |
| 24 h fresh | 1.70 | 1.80 | 106 |
| 48 h aged | 1.70 | 1.78 | 105 |
| 9.39 | 0 h fresh | 3.75 | 3.78 | 101 |
| 24 h aged | 3.75 | 3.48 | 93.0 |
| 24 h fresh | 3.75 | 3.74 | 100 |
| 48 h aged | 3.75 | 3.91 | 104 |
| 20.7 | 0 h fresh | 8.26 | 8.23 | 100 |
| 24 h aged | 8.26 | 8.38 | 101 |
| 24 h fresh | 8.26 | 8.61 | 104 |
| 48 h aged | 8.26 | 8.89 | 108 |
| 45.5 | 0 h fresh | 18.2 | 18.8 | 103 |
| 24 h aged | 18.2 | 18.1 | 99.0 |
| 24 h fresh | 18.2 | 18.7 | 103 |
| 48 h aged | 18.2 | 19.0 | 104 |
| 100 | 0 h fresh | 39.9 | 39.8 | 100 |
| 24 h aged | 39.9 | 39.4 | 99.0 |
| 24 h fresh | 39.9 | 39.5 | 99.0 |
| 48 h aged | 39.9 | 39.8 | 100 |

Limit of quantification (LOQ) = 0.170 mg/L. Limit of detection (LOD) = 0.0468 mg/L.

Table A 10: Measured concentrations of ametoctradin in test water

| **Test item [mg product/L]** | **Timepoint** | **Nominal Concentration [mg a.s./L]** | **Measured Concentration [mg a.s./L]** | **Recovery [%]** |
| --- | --- | --- | --- | --- |
| Control | 0 h fresh | 0 | <LOD | - |
| 24 h aged | 0 | <LOD | - |
| 24 h fresh | 0 | <LOD | - |
| 48 h aged | 0 | <LOD | - |
| 4.27 | 0 h fresh | 0.547 | 0.556 | 102 |
| 24 h aged | 0.547 | 0.408 | 75.0 |
| 24 h fresh | 0.547 | 0.588 | 107 |
| 48 h aged | 0.547 | 0.290 | 53.0 |
| 9.39 | 0 h fresh | 1.20 | 1.19 | 99 |
| 24 h aged | 1.20 | 0.544 | 45.0 |
| 24 h fresh | 1.20 | 1.22 | 102 |
| 48 h aged | 1.20 | 0.452 | 38.0 |
| 20.7 | 0 h fresh | 2.65 | 2.70 | 102 |
| 24 h aged | 2.65 | 1.37 | 52.0 |
| 24 h fresh | 2.65 | 2.72 | 103 |
| 48 h aged | 2.65 | 1.12 | 42.0 |
| 45.5 | 0 h fresh | 5.82 | 6.08 | 104 |
| 24 h aged | 5.82 | 3.18 | 55.0 |
| 24 h fresh | 5.82 | 6.06 | 104 |
| 48 h aged | 5.82 | 2.39 | 41.0 |
| 100 | 0 h fresh | 12.8 | 13.1 | 102 |
| 24 h aged | 12.8 | 8.24 | 64.0 |
| 24 h fresh | 12.8 | 13.0 | 102 |
| 48 h aged | 12.8 | 5.84 | 46.0 |

Limit of quantification (LOQ) =0.0547 mg/L, Limit of detection (LOD) = 0.0156 mg/L

1. **biological effects**

Following 48 hours of exposure, no immobilization of the test animals was observed in the control and up to and including the test item concentration of 100 mg product/L. After 24 hours no sublethal effects were observed in control and test item concentrations up to and including 100 mg product/L. After 48 hours two daphnids were observed to be sluggish in the test item concentration of 45.5 mg product/L. Biological results are presented in the following table.

Table A 11: Immobility of *Daphnia magna* exposed to BAS 743 02 F for 48 hours

|  |  |  |  |
| --- | --- | --- | --- |
| **Nominal Concentration [mg product/L]** | **No. of Daphnia tested** | **No. of Daphnia immobilised / (%)** | |
| **24 hours** | **48 hours** |
| Control | 20 | 0 / (0) | 0 / (0) |
| 6.25 | 20 | 0 / (0) | 0 / (0) |
| 12.5 | 20 | 0 / (0) | 0 / (0) |
| 25.0 | 20 | 0 / (0) | 0 / (0) |
| 50.0 | 20 | 0 / (0) | 0 / (0) |
| 100 | 20 | 0 / (0) | 0 / (0) |

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 12: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 202 (2004)** | **Obtained in this study** |
| In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised | 0% |
| The dissolved oxygen concentration at the end of the test should be 3 mg/l in control and test vessels | 7.91 mg O2/L in in all treatment groups |

1. **DEFICIENCIES**

There were no deviations with impact on quality and integrity of the study.

1. **Conclusion**

The acute toxicity of the test item BAS 743 02 F to *Daphnia magna* was investigated in a 48-hour semi-static test. The 48-hour EC50 of BAS 743 02 F to *Daphnia magna* was above the highest concentration tested of 100 mg product/L based on nominal concentrations. The NOEC was determined to be ≥ 100.0 mg product/L.

* + - 1. Study 3

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.2.1/03 |
| Report | Acute toxicity of BAS 743 03 F on *Daphnia magna* in a 48-hour static test  Renner, P., 2023  XXXX Study ID: 933750\_2  XXXX Doc ID: 2022/2033730 |
| Guidelines followed in study: | Biological part: OECD 202 (2004)  Analytical part: SANTE/2020/12830, Rev.1 (2021) |
| Deviations: | No |
| GLP/Officially recognised testing facilities: | Yes, GLP |
| Acceptability/Reliability: | Yes |

**Executive Summary**

The acute toxicity of the test item BAS 743 03 F to *Daphnia magna* was investigated in a 48-hour static test with the nominal concentrations 0 (control), 6.26, 12.5, 25.0, 50.0 and 100.0 mg porduct/L. Four replicates containing five Daphnia each were used for all treatment groups.

The 48-hour EC50 of BAS 743 03 F to *Daphnia magna* was above the highest concentration tested of 100 mg product/L based on nominal concentrations (99.6 mg product/L mean measured). Based on nominal concentrations, the NOEC was determined to be ≥ 100.0 mg product/L (99.6 mg product/L mean measured).

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. Test item: | BAS 743 03 F |
| Description: | Liquid, soluble concentrate (SC) |
| Lot/Batch: | FRE-002223 |
| Active substance content: | Propamocarb: nominal 378.0 g/L; analysed 376.7 g/L,  Ametoctradin: nominal 120.0 g/L; analysed 120.2 g/L |
| Density: | 1.071 g/cm3 |
| Storage conditions: | In original container, in a well-ventilated room, under cool and dry conditions in the dark |
| Stability (expiry date): | 31.01.2024 |
| 1. Control: | Untreated test medium |
| 1. Reference item: | Potassium dichromate was tested in a separate study to verify test system sensitivity. |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. Test organism: | *Daphnia magna* (Straus) |
| Age | < 24 hours |
| Source: | In-house laboratory culture, originally purchased from RWTH Aachen University, Institute for Environmental Research, Aachen, Germany |
| Culture medium: | M4 medium |
| Test medium: | Reconstituted water according to OECD 202 (ISO 6341) |
| Acclimatisation: | Brood Daphnia were maintained in test medium under test conditions prior to test start for 48 hours. |
| Diet: | No feeding during the test, before exposure fed with a defined suspension of green algae (*Desmodesmus subspicatus*) daily |
| 1. Test units: | 25 ml-glass beakers containing approx. 10.5mL of test medium, loosely covered with a plastic lid |
| 1. Environmental conditions |  |
| Temperature: | 19.8 – 20.0 °C |
| pH: | 7.98 – 8.13 |
| Dissolved O2 concentration: | 8.53 – 8.71 mg/L |
| Hardness: | 140 – 250 mg/L CaCO3 |
| Photoperiod: | 16 h light : 8 h dark |
| Light intensity: | 20 µE m-2 s-1 |
| Aeration of the test water: | No aeration of test vessels |

1. **Treatment:**

The study was conducted at BioChem agrar (Kupferstraße 6, Machern OT Gerichshain, Germany) between 31.01.2023 and 02.02.2023. The study was conducted as a static dose-response test with five test item nominal concentrations of 6.26, 12.5, 25.0, 50.0 and 100.0 mg product/L and one control with untreated test medium. The test media were prepared just before introduction of the daphnids (= start of the test). Four replicates containing five Daphnia each were used for all treatment groups and the control. The duration of the test was 48 hours.

1. **Dose preparation:**

A stock solution of 250.0 mg test item/L was prepared by dissolving 52.5 mg test item into 250 mL test water by intense stirring for 5 minutes. Adequate volumes of this stock solution were diluted with test water to prepare the test media of the desired test concentrations. The test media were prepared just before introduction of the daphnids (= start of the test).

The stock solutions appeared slightly turbid. Precipitation of any kind were not seen. Individual stock solutions per treatment group were prepared as bulk solutions. Test solutions appeared clear and transparent. Precipitation of any kind were not seen.

1. **Measurements and observations:**

Biological observations:

The mobility of the daphnids was determined by visual observation after 3, 24 and 48 hours. Those animals not able to swim within 15 seconds after gentle agitation of the test vessels were considered to be immobile. Any abnormal behaviour or appearance was recorded (e.g. trapping at surface of water or discoloration).

Physicochemical measurements:

At test start and test end, the pH and oxygen content in test solutions was recorded. The temperature was recorded continuously.

Analytical verification:

At test start, samples for analysis were taken from additional test vessels which were setup under identical conditions and did therefore contain Daphnia. Samples after 24 hours and test end were taken from the actual test vessels used in the test. 1 mL sampling vials were labelled, filled with 0.5 mL test solution and 0.5 mL acetonitrile/0.2% formic acid was added right after. All samples were stored deep frozen until usage during the analytical phase of the study.

Concentrations of the test item BAS 743 03 F were determined via its active substances propamocarb and BAS 650 F (ametoctradin) in test item solutions. The determination was conducted by using high performance liquid chromatography (HPLC) with mass-spectrometric (MS-MS) detection.

1. **Statistical analysis:**

ECx could not be quantified due to the absence of toxicity of the test item. Monotonicity (trend analysis by contrasts, p ≤ 0.05) was performed. The LOEC was determined using Fisher`s Exact Binomial Test with Bonferroni Correction (a = 0.05, one-sided greater). Statistics and modelling were done using ToxRat professional version 3.3.0 (RATTE, 2018).

1. **Description of the analytical procedures**

Concentrations of the test item BAS 743 03 F were determined via its active substances propamocarb and BAS 650 F (ametoctradin) in test item solutions. The determination was performed using HPLC-MS/MS.

For the specimens, which were present in reconstituted, no extraction was necessary. They were diluted with 50/50 (v/v) 0.2% formic acid in acetonitrile/ test matrix into the range of the calibration curve before injecting into the HPLC-system. The method has limits of quantification (LOQ) of 0.4882 mg/L (propamocarb) and 0.1550 mg/L (BAS 650 F). The limits of detection (LOD) were ≤ 30% of LOQ and are defined as the lowest calibration level for each sample matrix.

Stability samples prepared one day before test start were not analysed since analyses for propamocarb and BAS 650 F were conducted less than 30 days after test start. Blank control samples (blanks of the carrier without test item) were analysed within this study. No significant interference from the blank carrier within the test item could be observed.

A matrix matched calibration was performed from 6.574 ng/mL to 119.5 ng/mL propamocarb (corresponding to 0.1315 mg/L to 2.391 mg/L considering the lowest applied overall dilution factor (DFOverall) of 20 mL/mL) as well as 2.087 ng/mL to 37.95 ng/mL BAS 650 F (corresponding to 0.04174 mg/L to 0.7589 mg/L considering the lowest applied DFOverall of 20 mL/mL).

Results of the validation experiments obtained during analysis of BAS 743 03 F showed that the mean recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for propamocarb and BAS 650 F, with relative standard deviations RSD < 20%. No significant peak interferences (>LOD) occurred at the retention time and mass transition in the control samples.

The validity criteria (specificity, linearity, accuracy and precision) for the analytical method have been met according to SANTE/2020/12830, Rev.1 and ENV/JM/MONO(2007)17.

Table A 13: Procedural recoveries for Propamocarb and BAS 650 F applied as BAS 743 03 F in test water

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample Matrix** | **Fortification Level [mg/L]** | **n** | **Recoveries [%]** | **Mean Recovery [%]** | **RSD [%]** |
| Propamocarb | 0.000 | 2 | - | <LOD | - |
| 0.4882 | 5 | 99.9, 103, 99.0, 101, 101 | 101 | 1.39 |
| 24.41 | 5 | 98.7, 100, 94.3, 97.4, 98.0 | 97.7 | 2.17 |
| Overall | 10 | - | 99.1 | 2.31 |
| Ametoctradin | 0.000 | 2 | - | <LOD | - |
| 0.1550 | 5 | 101, 102, 98.3, 102, 98.6 | 100 | 1.83 |
| 7.749 | 5 | 98.7, 98.8, 97.3, 101, 97.8 | 98.8 | 1.57 |
| Overall | 10 | - | 99.6 | 1.82 |

1. **Results and Discussion**
2. **ANALYTICAL RESULTS**

The content of both active substances propamocarb and ametoctradin (BAS 650 F) in test solutions was analytically determined to confirm the correct application of the test item BAS 743 03 F. Recoveries of propamocarb and ametoctradin were within 80 to 120 % of nominal concentrations in fresh test solutions. In spent test solutions after 24 and 48 hours, recoveries of propamocarb were within 111 to 125 % of nominal concentrations while recoveries of ametoctradin were within 35.1 to 58.7 % of nominal concentrations. Based on these findings, toxicity results were related to test item nominal and test item mean measured concentrations. The analytical results are presented in the following tables.

Table A 14: Measured concentrations of propamocarb in test water

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test item [mg product/L]** | **Nominal Concentration [mg a.s./L]** | **Timepoint [hours]** | **Measured Concentration [mg a.s./L]** | **Recovery [%]** |
| Control | - | 0 | <LOD | - |
| 24 | <LOD | - |
| 48 | <LOD | - |
| 6.26 | 2.209 | 0 | 2.373 | 107 |
| 24 | 2.541 | 115 |
| 48 | 2.649 | 120 |
| 12.5 | 4.412 | 0 | 4.497 | 102 |
| 24 | 4.886 | 111 |
| 48 | 5.417 | 123 |
| 25.0 | 8.824 | 0 | 9.136 | 104 |
| 24 | 9.790 | 111 |
| 48 | 10.62 | 120 |
| 50.0 | 17.65 | 0 | 17.54 | 99.4 |
| 24 | 20.30 | 115 |
| 48 | 20.84 | 118 |
| 100.0 | 35.29 | 0 | 36.74 | 104 |
| 24 | 39.83 | 113 |
| 48 | 44.16 | 125 |

Limit of Quantification (LOQ) = 0.4882 mg/L Propamocarb

Table A 15: Measured concentrations of ametoctradin in test water

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test item [mg product/L]** | **Nominal Concentration [mg a.s./L]** | **Timepoint [hours]** | **Measured Concentration [mg a.s./L]** | **Recovery [%]** |
| Control | - | 0 | <LOD | - |
| 24 | <LOD | - |
| 48 | <LOD | - |
| 6.26 | 0.7014 | 0 | 0.6595 | 94.0 |
| 24 | 0.4115 | 58.7 |
| 48 | 0.4083 | 58.2 |
| 12.5 | 1.401 | 0 | 1.272 | 90.8 |
| 24 | 0.7445 | 53.2 |
| 48 | 0.7503 | 53.6 |
| 25.0 | 2.801 | 0 | 2.593 | 92.6 |
| 24 | 1.589 | 56.7 |
| 48 | 1.386 | 49.5 |
| 50.0 | 5.602 | 0 | 5.330 | 95.1 |
| 24 | 2.116 | 37.8 |
| 48 | 1.965 | 35.1 |
| 100.0 | 11.20 | 0 | 11.12 | 99.3 |
| 24 | 5.004 | 44.7 |
| 48 | 4.280 | 38.2 |

Limit of Quantification (LOQ) = 0.1315 mg/L Ametoctradin

1. **biological effects**

Following 48 hours of exposure, no immobilization of the test animals was observed in the control and up to and including the nominal test item concentration of 25 mg product/L. 5% immobilization was observed in the test concentrations of 50 and 100 mg test item/L after 48 h. During the 48-hour exposure, no discolorations nor symptoms of any abnormal behaviour were found. Biological results are presented in the following table.

Table A 16: Immobility of *Daphnia magna* exposed to BAS 743 03 F for 48 hours

|  |  |  |  |
| --- | --- | --- | --- |
| **Nominal Concentration [mg product/L]** | **No. of Daphnia tested** | **No. of Daphnia immobilised / (%)** | |
| **24 hours** | **48 hours** |
| Control | 20 | 0 / (0) | 0 / (0) |
| 6.25 | 20 | 0 / (0) | 0 / (0) |
| 12.5 | 20 | 0 / (0) | 0 / (0) |
| 25.0 | 20 | 0 / (0) | 0 / (0) |
| 50.0 | 20 | 0 / (0) | 1 / (5) |
| 100 | 20 | 0 / (0) | 1 / (5) |

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 17: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 202 (2004)** | **Obtained in this study** |
| In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised | 0% |
| The dissolved oxygen concentration at the end of the test should be 3 mg/l in control and test vessels | 8.53 mg O2/L in in all treatment groups |

Daphnia were not trapped at the water surface, as required.

1. **DEFICIENCIES**

There were no deviations with impact on quality and integrity of the study.

1. **Conclusion**

The 48-hour EC50 of BAS 743 03 F to *Daphnia magna* was above the highest concentration tested of 100 mg product/L based on nominal concentrations (99.6 mg product/L mean measured). Based on nominal concentrations, the NOEC was determined to be ≥ 100 mg product/L BAS 743 03 F (99.6 mg/L mean measured).

* + - 1. Study 4

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guideline 201 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.2.1/04 |
| Report | BAS 743 02 F: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindák under Laboratory Conditions  Obert-Rauser, P., 2023  XXXX Study ID: 933752\_5  XXXX Doc ID: 2022/2033713 |
| Guideline(s): | Biological part: OECD 201 (2011)  Analytical part: SANCO/3029/99 rev.4 11/07/00 |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The toxicity of the test item BAS 743 02 F to *Pseudokirchneriella subcapitata* was investigated in a 72-hour static test with the nominal concentrations 0 (control), 0.954, 3.05, 9.77, 31.3 and 100 mg product/L. There were five replicates for each test item concentration and ten replicates for the untreated control. Cell density was determined by fluorescence detection after 24, 48 and 72 hours of exposure. Biological results are related to nominal concentrations.

The ErC10-, ErC20- and ErC50-value and the EyC50-value were considered to be > 100 mg product/L. Due to an inhibition of yield below 30 % and a missing concentration response relation, no reliable values were calculable and the EyC10, 20-values were not determined. The overall LOEC was determined to be 100 mg product/L and the corresponding NOEC was set at 31.3 mg product/L.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Description:** | White liquid, soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L; Propamocarb, nominal 432.0 g/L, analysed 431.0 g/L |
| **Density:** | 1.080 g/cm3 |
| **Storage conditions:** | 5 °C – 30 °C , dark and dry |
| **Stability (expiry date):** | 31.01.2024 |
| 1. **Control:** | Untreated test medium (AAP medium) |
| 1. **Reference item:** | Potassium dichromate is tested as the toxic reference item in a separate study twice a year to confirm the sensitivity of the test organism. |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. **Test organism:** | *Pseudokirchneriella subcapitata* (Korshikov), Strain No. 61.81 SAG |
| **Source:** | Commercial supplier, MBM Sciencebridge GmbH, Göttingen, Germany |
| **Culture medium:** | AAP-Medium |
| **Acclimatisation:** | Test algal cells were taken from an exponentially growing pre-culture, which was set up 3 – 4 days prior to the test start under the same conditions as in the test. |
| **Initial cell density:** | 0.5 × 104 cells/mL |
| 1. **Test units:** | Erlenmeyer flasks of 100 mL volume with approximately 50 mL of test medium |
| 1. **Environmental conditions** |  |
| **Temperature:** | 22.4 – 23.6 °C |
| **pH:** | 7.31 – 7.35 at test start, 7.65 to 8.83 at test end |
| **Dissolved O2 concentration:** | Not reported |
| **Hardness:** | Not reported |
| **Photoperiod:** | Continuous illumination |
| **Light intensity:** | 96.3 µEm-2s-1 |

1. **Treatment:**

The study was conducted at Eurofins Aquatic Ecotoxicology GmbH (Niefern-Öschelbronn, Germany) between 28.11.2022 and 13.12.2022. The study was conducted as a static dose-response test with five test item concentrations (0.954, 3.05, 9.77, 31.3 and 100 mg product/L) and one control with untreated test medium. Non-GLP pre-experiments were performed to determine a suitable concentration range.

The test was started (0 hours) by inoculation of a biomass of nominal 5000 algal cells per mL test medium. These cells were taken from an exponentially growing pre-culture, which was set up 3 to 4 days prior to the test start under the same conditions as in the test. Volumes of 50 mL of algal suspension per replicate were placed in an incubator in 100 mL Erlenmeyer flasks on a pivoted bogie which turns around and induces shaking by regular sudden stops. Ten replicates were used for the control and five for each test item concentration. The duration of this dose-response test was 72 hours.

1. **Dose preparation:**

The necessary amount of test item for preparing the 100 mg/L stock solution was weighed into a vial. Test medium was added and the solution was homogenised by shaking. Lower concentrated stock solutions were prepared by dilution of appropriate solutions with test medium. In this process a stock solution for each test item concentration was produced. Stock solutions appeared to be turbid, additionally in the stock solutions of concentrations from 9.77 mg/L upwards, frothing was observed. For the preparation of the test solutions, defined volumes of the prepared stock solutions were added to test medium followed by homogenization by shaking. The highest test solution was observed to be turbid, the test item concentration of 31.3 mg/L was observed to be lightly turbid and all lower concentrations were clear and transparent. All stock and test solutions were prepared with AAP-medium containing algae cells with a target density of 0.5 × 104 cells per mL.

1. **Measurements and observations:**

Biological observations:

At test start, the number of cells in each control replicate was determined in duplicate. At defined time periods (24, 48 and 72 hours), the number of cells in each replicate was determined in duplicate by fluorescence measurement. The fluorescence measurements were performed with a fluorescence microplate reader with an emission wavelength of 670 nm and evaluated with Tecan i-control (Software for Tecan Readers Tecan i-control, 2.0.10.0). By the means of a calibration curve, where fluorescence signals were plotted versus cell numbers, the cell numbers were derived from the fluorescence signals. To establish a calibration curve, the cell numbers were counted with a Neubauer chamber after preparation of a dilution series of a logarithmic growing *Pseudokirchneriella subcapitata* culture. Additionally, the morphological appearance of the algae cells was assessed microscopically at the end of the test.

Physicochemical measurements:

Measurements of pH-value were performed at test start and test end, the temperature was measured continuously and recorded at 0, 24, 48 and 72 hours. The light intensity of all positions of the incubator is measured once a year and was confirmed for one representative position at test start. Appearance of test solution was assessed and documented on a daily basis.

Analytical verification:

Analytical samples including retain samples were taken in the main test at 0 hours from fresh test solutions and after 24 hours and 72 hours from aged test solutions from all test item concentrations and control. For each sampling also two retain samples were taken. Additional samples were taken from the stock solution at test start. All samples were stored deep frozen (≤ -18 °C) until they were transferred to the analytical laboratory.

The analytical verification of test item concentrations in test solutions was done by analysing the content of ametoctradin and propamocarb in the samples taken during the test. The content of the analytes in the test solution samples was determined by analysing with HPLC-MS/MS. The analytical method was fully validated in accordance with the requirements of Guidance Document SANTE/2020/12830 Rev. 1 (2021).

1. **Statistical analysis:**

The NOEC and LOEC were determined by using a multiple comparison method (Dunnetts-t-test, left sided, for growth rate and yield). Due to a weak concentration response relation and since the inhibition was below 50 % at the highest test item concentration for both growth rate and yield, the data was inappropriate for statistical determination of the ECx-values. The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat® Solutions GmbH.

1. **Description of the analytical procedures**

XXXX analytical method APL0500/02 was modified for the analysis of ametoctradin and propamocarb. Sample analysis was performed by dilution of the sample with 50 µL formic acid and the equal amount of acetonitrile. Subsequently, the samples were diluted with acetonitrile/test medium + 0.5 % formic acid (1:1, v/v) to be within the range of the calibration curve. Final determination was accomplished by LC-MS/MS. The method has a limit of quantification (LOQ) of 1.20 ng/mL ametoctradin (corresponding to 0.0122 mg ametoctradin/L) and 3.60 ng/mL propamocarb (corresponding to 0.0381 mg propamocarb/L). The limit of detection (LOD) is of 0.360 ng/mL Ametoctradin (corresponding to 0.00360 mg ametoctradin/L) and 1.08 ng/mL propamocarb (corresponding to 0.0108 mg propamocarb/L).

Results of the validation and procedural recovery experiments obtained during analysis of showed that the recovery efficiency and repeatability was within the acceptable range of 70% to 120% of the intended concentrations for analyte BAS 743 02 F, with relative standard deviations RSD < 20%. No significant peak interferences occurred at the retention time and mass transitions of BAS 743 02 F. For ametoctradin, overall recoveries ranged from 89% to 103% for all fortification levels. For propamocarb, overall recoveries ranged from 101% to 111% for all fortification levels. The results obtained from measurements of the untreated control samples were below the LOD. Matrix-matched calibration standards were used for procedural recovery experiments and residue analysis.

The storage stabilities of ametoctradin and propamocarb were investigated by fortification of at least six untreated test medium with the test item. Three of the samples were analyzed on the day of fortification (day 0). The other fortified samples were stored frozen (≤ 18°C). After 15 days of storage, three samples were analyzed. This time period covers the longest storage period of samples of 12 days for test medium. The results show that both ametoctradin and propamocarb were stable under deep-frozen conditions (≤ 18 °C) in test medium for at least 15 days.

The validity criteria (specificity, linearity, accuracy and precision) for the analytical method have been met according to SANTE/2020/12830 Rev.

Table A 18: Procedural recoveries for ametoctradin and propamocarb applied as BAS 743 02 F in Test Water

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Analyte** | **Fortification Level (mg/L)** | **n** | **Recoveries (%)** | **Mean Recovery (%)** | **RSD (%)** |
| Ametoctradin | Control | 2 | <LOD | - | - |
| 0.0954 | 11 | 69.0, 74.0, 80.0, 93.0, 93.0, 94.0, 97.0, 100, 101, 96.0, 95.0 | 90.0 | 12.0 |
| 130 | 5 | 105, 101, 102, 104, 102 | 103 | 2.0 |
| **Overall** | **17** | **-** | **94.0** | **11.0** |
| Propamocarb | Control | 2 | <LOD | - | - |
| 0.0954 | 11 | 111, 108, 109, 110, 110, 118, 116, 117, 102, 104, 103 | 110 | 5.0 |
| 130 | 5 | 103, 100, 100, 102, 99.0 | 101 | 2.0 |
| **Overall** | **17** | **-** | **107** | **6.00** |

RSD = Relative standard deviation

1. **Results and Discussion**
2. **ANALYTICAL RESULTS**

The measured concentrations of ametoctradin in samples taken at test start ranged from 80 % to 102 % of nominal. In the samples taken from aged solutions, the measured concentrations were between 12 % and 77 % of nominal. The measured concentrations of propamocarb in samples taken at test start ranged from 105 % to 110 % of nominal. In the samples taken from aged solutions after 72 h, the measured concentrations were between 98 % and 109 % of nominal. The toxicological endpoints were evaluated using the nominal concentrations of the test item. The analytical results are presented in the following tables.

Table A 19: Measured concentrations of ametoctradin in the exposure solutions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test item nominal  [mg product/L]** | **Nominal Concentration [mg/ a.s.L]** | **Sampling** | **Measured Concentration** | |
| **[mg a.s./L]** | **% of nominal** |
| Control | 0 | 0 h fresh | < LOD | - |
| 24 h aged | < LOD | - |
| 72 h aged | < LOD | - |
| 0.954 | 0.122 | 0 h fresh | 0.0970 | 80 |
| 24 h aged | 0.120 | 98 |
| 72 h aged | 0.0875 | 72 |
| 3.05 | 0.390 | 0 h fresh | 0.348 | 89 |
| 24 h aged | 0.288 | 74 |
| 72 h aged | 0.127 | 33 |
| 9.77 | 1.25 | 0 h fresh | 1.18 | 94 |
| 24 h aged | 0.995 | 80 |
| 72 h aged | 0.151 | 12 |
| 31.3 | 4.01 | 0 h fresh | 4.04 | 101 |
| 24 h aged | 3.86 | 96 |
| 72 h aged | 2.44 | 61 |
| 100 | 12.8 | 0 h fresh | 13.1 | 102 |
| 24 h aged | 12.5 | 98 |
| 72 h aged | 9.85 | 77 |

- = not calculated; LOD = 0.00360 mg/L ametoctradin; LOQ = 0.0122 mg/L ametoctradin

Table A 20: Measured concentrations of propamocarb in the exposure solutions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test item nominal  [mg product/L]** | **Nominal Concentration [mg/ a.s.L]** | **Sampling** | **Measured Concentration** | |
| **[mg a.s./L]** | **% of nominal** |
| Control | 0 | 0 h fresh | < LOD | - |
| 24 h aged | < LOD | - |
| 72 h aged | < LOD | - |
| 0.954 | 0.381 | 0 h fresh | 0.400 | 105 |
| 24 h aged | 0.426 | 112 |
| 72 h aged | 0.377 | 99 |
| 3.05 | 1.22 | 0 h fresh | 1.29 | 106 |
| 24 h aged | 1.30 | 107 |
| 72 h aged | 1.33 | 109 |
| 9.77 | 3.90 | 0 h fresh | 4.02 | 103 |
| 24 h aged | 3.84 | 98 |
| 72 h aged | 3.84 | 98 |
| 31.3 | 12.5 | 0 h fresh | 13.2 | 106 |
| 24 h aged | 13.1 | 105 |
| 72 h aged | 13.3 | 106 |
| 100 | 39.9 | 0 h fresh | 43.8 | 110 |
| 24 h aged | 38.7 | 97 |
| 72 h aged | 39.4 | 99 |

= not calculated; LOD = 0.0108 mg/L propamocarb; LOQ = 0.0381 mg/L propamocarb

1. **biological effects**

After 72 hours, at termination of the test, no concentration response relation was observed for the inhibition of growth rate and yield. The inhibition of growth rate peaked at 5.4 % at a nominal test item concentration of 100 mg product/L and the inhibition of yield peaked at 22.6 % at a nominal test item concentration of 100 mg product/L. The morphology of the algae cells was investigated microscopically at test end. The cells were considered normal for the control and up to and including a nominal test item concentration of 100 mg product/L.

Significant inhibitory effects were determined for growth rate at test item concentrations of 0.954 and 100 mg product/L. Since no statistically significant inhibition was observed at concentrations from 3.05 to 31.3 mg product/L (missing concentration-response relation), the statistically significant inhibition at 0.954 mg product/L for growth rate is not considered as test item related.

For yield, significant inhibitory effects were determined at test item concentrations of 100 mg prodcut/L. The overall LOEC was therefore determined to be 100 mg product/L and the corresponding NOEC was set at 31.3 mg/L.

A summary of the effects on cell density, yield and growth rate is presented in the following tables.

Table A 21: Summary of effects on cell density

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Nominal concentration**  **[mg product/L]** | **Mean cell density [× 104 cells/mL]** | | | |
| **0 hour** | **24 hours** | **48 hours** | **72 hours** |
| Control | 0.55 | 2.24 | 13.74 | 67.42 |
| 0.954 | 0.55 | 2.17 | 11.89 | 58.69 |
| 3.05 | 0.55 | 2.40 | 15.34 | 75.55 |
| 9.77 | 0.55 | 2.44 | 16.69 | 75.37 |
| 31.3 | 0.55 | 2.35 | 15.87 | 73.09 |
| 100 | 0.55 | 1.84 | 11.43 | 52.29 |

Table A 22: Summary of effects on yield

|  |  |  |  |
| --- | --- | --- | --- |
| **Nominal concentration**  **[mg product/L]** | **Yield [× 104 cells/mL] [% inhibition]** | | |
| **0 - 24 hours** | **0 - 48 hours** | **0 - 72 hours** |
| Control | 0.0 | 0.0 | 0.0 |
| 0.954 | 4.1 | 14.0 | 13.1 |
| 3.05 | -9.5 | -12.1 | -12.2 |
| 9.77 | -11.8 | -22.4 | -11.9 |
| 31.3 | -6.5 | -16.1 | -8.5 |
| 100 | 23.7 | 17.5 | 22.6\* |

negative values in % inhibition indicate an increase in growth relative to that of the control

\* mean value significantly different from the control

Table A 23: Summary of effects on growth rate

|  |  |  |  |
| --- | --- | --- | --- |
| **Nominal concentration [mg test item/L]** | **Growth rate [1/day] [% inhibition]** | | |
| **0-24 hours** | **0-48 hours** | **0-72 hours** |
| Control | 0.0 | 0.0 | 0.0 |
| 0.954 | 3.3 | 5.0 | 3.2\* |
| 3.05 | -4.5 | -3.2 | -2.3 |
| 9.77 | -5.7 | -5.9 | -2.2 |
| 31.3 | -3.1 | -4.3 | -1.5 |
| 100 | 14.3 | 5.9 | 5.4\* |

negative values in % inhibition indicate an increase in growth relative to that of the control

\* Statistically significant difference to the control; since no statistically significant inhibition was observed at concentrations from 3.05 to 31.3 mg product/L (missing concentration-response relation), the statistically significant inhibition at 0.954 mg/L is not considered as test item related

Table A 24: Summary of endpoints

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Growth rate** | **Yield** |
| **[mg product/L] nom** | **[mg product/L] nom** |
| 72-hour EC50 (95% conf. interval) | >100 (>100) 1 | >100 (>100) 3 |
| 72-hour EC20 (95% conf. interval) | >100 (>100) 1 | -2 |
| 72-hour EC10 (95% conf. interval) | >100 (>100) 1 | - 2 |
| 72-hour NOEC | 31.3 4 | 31.3 4 |
| 72-hour LOEC | 100 4 | 100 4 |

1) Inhibition at highest test item concentration was below 10 %, therefore the ErC10, ErC20 and ErC50-values are considered to be above the highest test item concentration

2) Due to an inhibition below 30 % and a weak concentration response relation the database was inappropriate for statistical determination of the EyCx-values, which hence was not performed.

3) Inhibition at highest test item concentration was below 30 %, therefore the EyC50-value is considered to be above the highest test item concentration

4) Following Dunnett’s t-test (left-sided, p≤0.05) for growth rate and yield

Potassium dichromate is tested as the toxic reference item in a separate study twice a year to confirm the sensitivity of the test organism against compounds with known effects under the test conditions. The ErC10 and the EyC10 were determined to be 0.516 and 0.175 mg/L, respectively (nominal). The ErC20 and EyC20) were determined to be 0.746 and 0.288 mg/L, respectively (nominal). The ErC50 and EyC50 were determined to be 1.30 mg/L and 0.611 mg/L, respectively (nominal). Therefore, the EC50 values calculated in this reference test were considered to be within an acceptable range and it can be considered that the test organism is sensitive.

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 25: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 201 (2011)** | **Obtained in this study** |
| The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. | 122.58-fold increase within 72 hours |
| The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. | 13 % |
| The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. | 2.3 % |

1. **DEFICIENCIES**

There were no deviations with impact on quality and integrity of the study.

1. **Conclusion**

The toxicity of the test item BAS 743 02 F to *Pseudokirchneriella subcapitata* was investigated in a 72-hour static test with the nominal concentrations 0 (control), 0.954, 3.05, 9.77, 31.3 and 100 mg product/L. Biological results are related to nominal and geometric mean measured concentrations.

The ErC10-, ErC20- and ErC50-value and the EyC50-value were considered to be > 100 mg product/L. Due to an inhibition of yield below 30 % and a missing concentration response relation, no reliable values were calculable and the EC10, 20-values for yield were not determined. The overall LOEC was determined to be 100 mg product/L and the corresponding NOEC was set at 31.3 mg product/L.

* + 1. KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms
       1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OCSPP Guideline 850.1350 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.2.2/01 |
| Report: | BAS 650 F - Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) Following Draft OPPTS Guideline 850.1350  Schwader. A.L., 2013  XXXX Study ID: 249289  XXXX Doc ID: 2013/7000443 |
| Guideline(s): | OCSPP Guideline 850.1350 (1996) (draft) |
| Deviations: | No |
| Previous evaluation: | Not previously evaluated |
| GLP/Officially recognised testing facilities: | Yes, conducted under GLP/Officially recognised testing facilities |
| Acceptability: | Yes |

**Executive summary**

The toxicity of BAS 650 F to the mysid (*Americamysis bahia*) over a full life-cycle was assessed over 28 days under flow through conditions at five nominal test item concentrations of 6.3, 13, 25, 50 and 100 µg a.s./L, corresponding to 4.7, 10, 18, 38 and 77 µg a.s./L mean measured concentration. An untreated dilution water control was tested in parallel. Four replicates were set up per treatment group. Observations were performed daily for mortality and abnormal appearances and behaviour. Reproductive success (total number of offspring produced per female) was determined along with survival of the second generation (F1). Body length and dry weight were measured in all mysids at test termination.

The mean measured concentrations during the exposure period ranged between 71 – 79% of nominals. The concentration which defined the NOEC (18 μg a.s./L) had an overall CV of 20%. Results of the study are based on mean measured concentrations.

Based on mean measured concentrations of BAS 650 F and male total body length (the most sensitive indicator of toxicity), the No-Observed-Effect Concentration (NOEC) was determined to be 18 μg a.s./L. The Lowest-Observed-Effect Concentration (LOEC) for mysids was determined to be 38 μg a.s./L. Since no concentration tested resulted in ≥ 50% mortality, the 7, 14, 21 and 28-day LC50 values were empirically estimated to be > 77 μg a.s./L, the highest mean measured BAS 650 F concentration tested.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 650 F (Ametoctradin) |
| **Description:** | Not reported |
| **Lot/batch:** | COD-000748 |
| **Purity:** | 99.3% |
|  |  |
| 1. **Control:** | Untreated dilution water |

1. **STUDY DESIGN AND METHODS**

|  |  |
| --- | --- |
| 1. **Test species:** | Mysid shrimp (*Americamysis bahia*) |
| **Age:** | ≤23.5 hours old |
| **Source:** | In-house culture |
| **Diet:** | Live brine shrimp (*Artemia salina*) nauplii, ≤48 hours old, provided twice daily |
| **Loading:** | ≤0.0025 g/L flowing solution/day |
|  |  |
| 1. **Test units:** | Glass test aquaria measuring 30 x 15 x 20 cm with a 10 cm high side drain that maintained a constant exposure solution volume of approximately 4.5 L.  Retention chambers: glass petri dishes, 10 cm diameter, 2 cm deep, with a 14 cm high Nitex screen collar (350 µm mesh size opening) attached with silicone sealant. Contained approx. 785 mL solution volume.  Pairing chambers: 6 cm diameter petri dishes, with a 14 cm high Nitex screen collar (350 µm mesh size opening) attached with silicone sealant. Contained approx. 250 mL solution volume. |
|  |  |
| 1. **Dilution water:** | Dilute, filtered, natural seawater |
| **Salinity:** | 20 – 21% |
| **pH:** | 7.7 – 7.9 |
| **Total organic carbon:** | 1.1 – 0.96 mg/L |
|  |  |
| 1. **Environmental conditions** |  |
| **Temperature:** | 25 – 27ºC |
| **pH:** | 7.7 – 8.0 |
| **Dissolved oxygen:** | 5.00 – 7.64 mg/L (70.7 – 104% saturation) |
| **Photoperiod:** | 16 h light : 8 h dark (760 - 1000 lux) |

1. **Test organism and treatment:**

Based on the results of a preliminary range finding test, five nominal test item concentrations of 6.3, 13, 25, 50 and 100 µg a.s./L, corresponding to 4.7, 10, 18, 38 and 77 µg a.s./L mean measured concentration, were selected for use in the study. An untreated dilution water control was tested in parallel. Four replicates were set up per treatment group, each containing 20 mysids (i.e. 80 mysids per treatment level and control), which were impartially selected and randomly distributed. Mysids were exposed to either the test item or the control for 28 days under flow-through conditions.

Prior to exposure initiation, an FLUID Metering, Inc (FMI) pump was calibrated to deliver 3.88 L/cycle of the 100 μg/L saturator column solution to the diluter system's mixing chamber. The mixing chamber was positioned over a magnetic stir plate which aided in the mixing of the stock solution. The solution contained in the mixing chamber constituted the highest nominal test concentration (at the functional solubility, expected to be approximately 100 μg a.s./L) and was subsequently diluted (50%) to provide the remaining nominal exposure concentrations (approximately 50, 25, 13 and 6.3 μg a.s./L).

Mysids, ≤ 23.5 hours old, were divided among 24 unlabelled beakers. The beakers contained culture water and were held in a water bath maintained at approximately 25ºC. The organisms were impartially selected and distributed to the beakers by adding five organisms at a time to each beaker until all beakers contained five mysids. This process was repeated until each beaker contained 20 mysids. Each group of 20 mysids was then impartially transferred to one of the 24 labelled retention chambers. The exposure was initiated when the retention chambers were placed in their respective exposure aquaria. Each exposure aquarium contained one retention chamber, yielding 20 mysids per replicate vessel and 80 organisms for each treatment level and the control.

When sexual maturity was reached (day 12) one mature male and one mature female were randomly assigned to each of the pairing chambers (with a maximum of five male/female pairs per replicate).

The exposure period lasted for a total duration of 28 days. At the end of the test, mysids were euthanised before final measurements were taken.

1. **Measurements and observations:**

For the F0 generation, the numbers of dead and living organisms were counted daily, and observations were made for abnormal appearances or behaviour. At the time an F1 generation pairing chamber was established and daily thereafter for 96 hours, observations of stress, abnormal behaviour (including discoloration, immobilisation and inability to maintain position in the water column), and survival were made.

At test termination, mysids were euthanised, removed from the test units, blotted dry and separated by sex for each replicate exposure level. A digital photograph was taken of each mysid for individual body length measurements.

Mysids were then dried in an oven at 60ºC for approximately 96 hours and then placed in a desiccator. Individual dry body weights were determined.

Reproductive success was calculated for each replicate aquarium as the total number of offspring produced per female. In addition, the percentage of actively reproducing females in each replicate of each treatment and the control was determined.

Temperature, dissolved oxygen, pH and salinity were measured in each replicate on day 0 and alternated between replicates daily thereafter throughout the exposure period for each treatment and control. Exposure solution temperature was also measured continuously in one control vessel.

Prior to the start of the definitive exposure, samples were removed from one replicate of each treatment level and the control and analysed for BAS 650 F concentration. In addition, a sample of the saturator column solution was also analysed during the pre-test period.

During the in-life phase of the study, samples were removed from alternating replicate solutions of each treatment level and control on days 0, 7, 8, 14, 21 and 28 for analysis of BAS 650 F concentrations. All samples were analysed using high pressure liquid chromatography with ultraviolet detection (HPLC/UV).

1. **Statistical analysis:**

The endpoints used for determination of significant adverse effect on F0 organisms included 28-day survival, male and female survival, growth (average dry body weight and average total body length) of both male and female mysids and reproduction (number of young released per female). The assumption that observations are normally distributed and homogeneous must be validated before parametric procedures can be performed. If the data are not normally distributed, then a non-parametric procedure is used for subsequent analyses. All endpoints were assessed in this fashion with the exception of survival endpoints (e.g., 28-day survival, male and female survival and F1 survival). Since the survival endpoints are binominal data, the Shapiro-Wilk’s test was used to check data for normality and the Bartlett’s test was used to check data for homogeneity of variance. All endpoints met the assumptions of normal distribution and homogeneity, therefore the Dunnett’s T3 Multiple Comparison Test or Dunnett's Multiple Comparison Test was used to evaluate the data. CETISTM (Ives, 2011) was used to perform the statistical computations.

1. **Description of the analytical procedures**

Methodology was validated to quantify the amount of BAS 650 F present in recovery samples prepared in filtered seawater. Recovery samples were diluted to a final composition of 20:80 acetonitrile:water and analyzed by automated injection on a high-performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV). This method was validated by fortification of filtered seawater with BAS 650 F at concentrations of 0.00500, 0.100 and 1.00 mg a.s./L. Three replicate dilutions were analyzed for each selected application solution. Recoveries averaged 99.1% ± 8.91% with a limit of quantitation of 0.00130 mg a.s./L. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 80 to 120%. The mean recovery was 99.1% with a standard deviation of 8.91%. The limit of quantitation was 0.00130 mg a.s./L.

1. **RESULTS AND DISCUSSION**
2. **BIOLOGICAL EFFECTS**

No behavioural abnormalities were observed during the exposure period at any test item concentration. At test termination, Fisher’s Exact Test with Bonferroni-Holm’s Adjustment determined no significant difference in male and female survival among organisms exposed to any of the treatment levels tested compared to the control data. Results are summarised in Table A 26.

Table A 26: Summary of the first generation (F0) survival at termination

|  |  |  |  |
| --- | --- | --- | --- |
| **Mean measured concentration (µg a.s./L)** | **Mean male survival ± SD (%)a** | **Mean female survival ± SD (%)a** | **Mean 28-day survival ± SD (%)b** |
| Control | 80 ± 18 | 86 ± 16 | 77 ± 16 |
| 4.7 | 89 ± 9 | 91 ± 10 | 77 ± 16 |
| 10 | 73 ± 9 | 78 ± 18 | 60 ± 7 |
| 18 | 77 ± 12 | 81 ± 5 | 70 ± 10 |
| 38 | 65 ± 27 | 76 ± 9 | 58 ± 15 |
| 77 | 94 ± 13 | 93 ± 8 | 78 ± 12 |

a Calculations of male and female survival began after pairing (day 12)

b 28-day survival is the overall survival throughout the exposure and does not differentiate by gender.

SD: standard deviation

Following 28 days of exposure, no significant difference in mean percentage of females producing young and mean number of offspring per female were determined when compared to the control Results are presented in Table A 27.

Table A 27: Summary of the first generation (F0) reproductive success (offspring per female) at termination

| **Mean measured concentration (µg a.s./L)** | **Mean % of females producing young ± SD** | **Mean no. offspring per female ± SD** |
| --- | --- | --- |
| Control | 90 ± 12 | 21.1 ± 5.0 |
| 4.7 | 100 ± 0 | 22.9 ± 2.4 |
| 10 | 100 ± 0 | 25.0 ± 2.4 |
| 18 | 95 ± 10 | 21.8 ± 5.7 |
| 38 | 88 ± 25 | 18.7 ± 8.8 |
| 77 | 100 ± 0 | 16.2 ± 1.7 |

SD: standard deviation

There was a statistically significant difference in the total body length of male mysids exposed to the 38 and 77 μg a.s./L treatment levels compared to the control data.

There was a statistically significant difference in dry body weight of male mysids at 77 µg a.s./L when compared to the control, and in females at 210 µg a.s./L. Results are presented in Table A 28.

Table A 28: Summary of mean total body length and dry body weight measurements of first generation (F0) mysids at termination

| **Mean measured concentration (µg a.s./L)** | **Mean total body length (mm) ± SD** | | **Mean dry body weight (mg) ± SD** | |
| --- | --- | --- | --- | --- |
| **Males** | **Females** | **Males** | **Females** |
| Control | 7.40 ± 0.10 | 7.48 ± 0.11 | 0.89 ± 0.05 | 1.20 ± 0.08 |
| 4.7 | 7.32 ± 0.07 | 7.67 ± 0.10 | 0.89 ± 0.01 | 1.35 ± 0.09 |
| 10 | 7.50 ± 0.18 | 7.69 ± 0.19 | 0.94 ± 0.10 | 1.34 ± 0.12 |
| 18 | 7.35 ± 0.17 | 7.56 ± 0.13 | 0.92 ± 0.02 | 1.27 ± 0.11 |
| 38 | 7.12 ± 0.16\* | 7.63 ± 0.13 | 0.82 ± 0.05 | 1.15 ± 0.07 |
| 77 | 7.04 ± 0.16\* | 7.40 ± 0.11 | 0.77 ± 0.04\* | 1.06 ± 0.07 |

\* Significantly reduced compared to the control, based on Dunnett’s Multiple Comparison Test.

SD: standard deviation

There was no statistically significant difference in F1 mysid survival among organisms exposed to any of the test item treatment levels, when compared to the control. Results are presented in Table A 29.

Table A 29: Summary of second generation (F1) survival at 96 hours post-release

|  |  |
| --- | --- |
| **Mean measured concentration (µg a.s./L)** | **Survival (%) ± SD** |
| Control | 100 ± 0 |
| 4.7 | 93 ± 5 |
| 10 | 98 ± 5 |
| 18 | 95 ± 6 |
| 38 | 98 ± 5 |
| 77 | 100 ± 0 |

SD: standard deviation

1. **ANALYTICAL RESULTS**

Due to the decrease in test concentrations on day 7, the test system was sampled on day 8 to confirm the recoveries. Measured concentrations of BAS 650 F were generally consistent between sampling intervals and the expected concentration gradient (50% dilution series) was maintained throughout the exposure. The concentration which defined the NOEC (18 μg a.s./L) had an overall CV of 20%. Results of the study are based on mean measured concentrations. Analysis of the quality control (QC) samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 89.1 to 104% (N = 15) of the nominal fortified levels (5.00, 25.0 and 100 μg/L). Based on these results, it was determined that satisfactory precision and quality control were maintained during the analysis of the exposure solutions. Analytical results are presented in Table A 30.

Table A 30: Summary of analytical results

| **Nominal concentration (µg/L)** | **Mean measured concentration during the 28-day life-cycle exposure (mg/L)** | **% of nominal** |
| --- | --- | --- |
| Control | n.a. | n.a. |
| 6.3 | 4.7 | 75 |
| 13 | 10 | 79 |
| 25 | 18 | 71 |
| 50 | 38 | 77 |
| 100 | 77 | 77 |

n.a.: not applicable

1. **VALIDITY CRITERIA**

At test termination, the mysids in the control met the performance criteria of the OPPTS 850.1350 guideline (> 70% survival of F0 mysids between pairing and exposure termination, > 75% of the females in the control released young and the control organisms produced > 3 offspring per female). Control mean survival between pairing and exposure termination was 77%; 90% of control females produced young, and control reproduction averaged 21.1 offspring per female.

1. **DEVIATIONS**

The protocol states that the organisms will be dried for approximately 24 hours at approximately 100 °C. For this test, the organisms were dried in an oven at approximately 60 °C for 92.5 hours. Since all replicates were treated equally and the control dry weight data compares to previous mysid life cycle studies, it can be assumed that this deviation did not have a negative impact on the results or interpretation of the study.

**III. Conclusion**

The toxicity of BAS 650 F to the mysid (*Americamysis bahia*) over a full life-cycle was assessed over 28 days under flow through conditions. Results of the study are based on mean measured concentrations. The NOEC was determined to be 18 µg a.s./L and the LOEC was determined to be 38 µg a.s./L. The MATC was determined to be 26 µg a.s./L.

* + 1. KCP 10.2.3 Further testing on aquatic organisms

Based on the outcome of the first-tier risk assessments, no further testing on aquatic organisms was considered necessary.

* 1. KCP 10.3 Effects on arthropods
     1. KCP 10.3.1 Effects on bees
        1. KCP 10.3.1.1 Acute toxicity to bees
           1. KCP 10.3.1.1.1 Acute oral toxicity to bees

Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 213 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.1.1.1/01 |
| Report | Acute toxicity of BAS 743 02 F to the honeybee *Apis mellifera* L. under laboratory conditions  Poráczki, K., 2023  XXXX Study ID: 933752\_1  XXXX Doc ID: 2022/2033708 |
| Guideline(s): | OECD 213: Honeybees, Acute Oral Toxicity Test (1998), OECD 214: Honeybees, Acute Contact Toxicity Test (1998) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The acute contact and oral toxicity of BAS 743 02 F was tested on honeybees (*Apis mellifera* L.) under laboratory conditions over 48 hours. Nominal test doses were 62.5, 125, 250, 500 and 1000 µg product/bee in both tests, that is corresponding to 527.0, 263.5, 131.7, 65.9 and 32.9 μg total a.s./bee, respectively (based on sum of nominal content of a.s.). Control groups were run in parallel (50% (w/v) sucrose solution in the oral test and a water and a wetting agent control in the contact test). Every treatment group in both tests comprised 3 replicates, with 10 bees per replicate.

In the contact toxicity test, no mortality occurred in the control groups either treated with deionised water or 1 % v/v tween solution. In the test item treatment, no mortality was observed after thoracic application of ≤ 1000 μg BAS 743 02 F/bee, after 48 hours. The LD50 (48 h) was > 1000 μg BAS 743 02 F/bee, corresponding to > 527.0μg total a.s./bee.

In the oral toxicity test, no mortality occurred in the control group fed with pure 50 % w/v sucrose solution. In the test item treatment, statistically significant mortality of 20.0 % was observed after oral consumption of 844 μg BAS 743 02 F/bee, after 48 hours. The LD50 (48 h) was > 816 μg BAS 743 02 F/bee, corresponding to > 444.7μg total a.s./bee.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| Description: | Liquid (colour not reported) |
| Lot/Batch: | FRE-002224 |
| Active substance content: | Ametoctradin (BAS 650 F), nominal 137.14 g/L, measured 137.7 g/L;  Propamocarb, nominal 432.0 g/L, measured 431.0 g/L |
| Density: | 1.080 g/cm³ (at 20°C) |
| 1. **Control:** | Oral test: 50% (w/v) sucrose solution  Contact test: Control 1: deionised water; Control 2: deionised water + 1% v/v wetting agent Tween 80 |
| 1. **Reference item:** | Dimethoate EC 400 (BAS 152 11 I) (401.7 g/L analysed) |
|  |  |

**B. STUDY DESIGN AND METHODS**

|  |  |
| --- | --- |
| 1. **Test species:** | Honeybee *Apis mellifera* L. Buckfast (Insecta, Hymenoptera, Apoidea) |
| **Age/life stage:** | Young adult female worker bees (3-5 weeks old) |
| **Source:** | BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany |
| **Diet:** | 50% w/v sucrose solution provided after application (immediately after thoracic application in the contact test and after removal of application feeders in the oral test). Food was provided *ad libitum*. |
| 1. **Test units:** | Disposable cardboard cages 95 mm x 50 mm x 65 mm (length x width x height) with holes in the bottom for ventilation and a glass plate at the front |
| 1. **Environmental conditions** |  |
| **Temperature:** | 24.2 – 24.4°C |
| **Relative humidity:** | 59.1 – 62.9% |
| **Photoperiod:** | 24-hour darkness (except for diffuse artificial light during assessments and handling) |

1. **Test organism and treatment:**

Both tests were performed as dose-response tests with 3 replicates per treatment group, each comprising one cage containing 10 bees.

1. **Oral toxicity test**

The bees were starved for 60 minutes before the test. At the start of the test, a quantity of 200 µL (corresponding to 0.2376 g) of the test item solution was offered to each cage, equivalent to 20 µL/bee. The test substance and toxic reference were administered in 50% w/v sucrose solution at the doses outlined in Table A 31.

Honeybees of a distinct group were assumed to consume approximately the same amount of the test item due to their social feeding behaviour. Bees in the control group were fed untreated 50% w/v sucrose solution.

Feeding tubes were weighed prior to addition of the sucrose solution. The feeders containing the appropriate diet were introduced through a hole in the roof of the cage. The feeding tubes were visibly empty approximately 3.5 hours after application. All feeding tubes were reweighed to determine the exact quantity of the test solution consumed and were then replaced with feeding tubes containing untreated 50% w/v sucrose solution.

Table A 31: Applied and consumed dosages in the oral toxicity test

| **Treatment group** | **Item applied** | **Applied dosages** | | **Actual uptake of the applied item** | |
| --- | --- | --- | --- | --- | --- |
| Control | Sucrose solution1 | -- | | -- | |
|  | | **(µg product/bee)** | **(µg total a.s./bee)** | **(µg product/bee)** | **(µg total a.s./bee)** |
| Test item | BAS 743 02 F\* | 62.5 | 32.9 | 62.5 | 32.9 |
| 125 | 65.9 | 125 | 65.7 |
| 250 | 131.7 | 249 | 131.2 |
| 500 | 263.5 | 487 | 256.8 |
| 1000 | 527.0 | 844 | 444.7 |
| Reference item | Dimethoate EC 400\*\* | 0.144 | 0.054 | 0.144 | 0.054 |
| 0.240 | 0.090 | 0.240 | 0.090 |
| 0.401 | 0.150 | 0.400 | 0.149 |
| 0.668 | 0.250 | 0.667 | 0.249 |

\* based on sum of nominal content of a.s.

\*\* based on analysed content of a.s.

1 respective solvent control for the test item and reference item treatment

Calculations are performed with non-rounded values

1. **Contact toxicity test**

Bees in the test cage were anaesthetised with CO2 for approximately 30 seconds and placed into a petri dish prior to application. A single 2 μL droplet of either the test item, control or toxic reference solution was placed on the dorsal bee thorax of each bee using an Eppendorf Micropipette. 1% Tween 80 solution was used as a vehicle to ensure a good penetration or adhesion of the droplet on the bee body. Tween solution is non-toxic to honeybees at the concentration used. Doses used for the test item and reference item are outlined in Table A 32.

Table A 32: Applied dosages in the contact toxicity test

| **Treatment group** | **Item applied** | **Dose rates** | |
| --- | --- | --- | --- |
| Control | Deionised water | -- | |
| Tween solution | **--** | |
|  | | **(µg product/bee)** | **(µg total a.s./bee)** |
| Test item | BAS 743 02 F\* | 62.5 | 32.9 |
| 125 | 65.9 |
| 250 | 131.7 |
| 500 | 263.5 |
| 1000 | 527.0 |
| Reference item | Dimethoate EC 400\*\* | 0.282 | 0.250 |
| 0.376 | 0.187 |
| 0.501 | 0.141 |
| 0.668 | 0.105 |

\* based on sum of nominal content of a.s.   
\*\* based on analysed content of a.s.

1 respective solvent control for the test item and reference item treatment

Calculations are performed with non-rounded values

1. **Dose preparation:**

**Oral test**

0.500 g BAS 743 02 F was weighed out and filled up to 10 mL with 50% w/v sucrose solution which gave a 5.000 % w/v stock solution. This was then diluted sequentially to produce the remaining 4 doses. The application volume was 200 µL/cage (equivalent to 20 µL per bee).

**Contact test**

5.000 g BAS 743 02 F was weighed out and filled up to 10 mL with 1% v/v Tween solution which gave a 50.000 % (w/v) stock solution. This was then diluted to produce the remaining 4 doses.

1. **Measurements and observations:**

In both tests, assessments of bee mortality and behavioural effects were made after 4, 24 and 48 hours. During the behavioural assessments, bees were classified as either healthy (normal), affected (impaired locomotion) or moribund. Other effects such as abnormal quantity/colour of excretion were also observed and recorded. All evaluations were made in comparison with the controls.

1. **Statistical analysis:**

For statistical evaluation of the mortality results the Fisher’s Binomial Test with Bonferroni-Holm correction or Step-down Cochran-Armitage Test procedure was used. The accepted significance level was p ≤0.05 (one-sided greater). The median lethal doses (LD50) along with the 95 % confidence limits were calculated with Probit analysis, using linear maximum likelihood regression for evaluation of the reference item, only.

The statistical calculations were performed with the computer program ToxRat Professional 3.3.0. (2018)

**II. RESULTS AND DISCUSSION**

**C. BIOLOGICAL EFFECTS**

**Oral test**

No mortality occurred in the control group fed with pure sucrose solution, after 48 hours. In the test item treatment, statistically significant mortality of 20.0 % was observed after oral consumption of 816 μg BAS 743 02 F/bee after 48 hours. Therefore, the LD50 (48 h) was > 844 μg consumed product/bee that is corresponding to > 444.7 μg total a.s./bee. No behavioural impairments were observed after oral application of ≤ 816 μg BAS 743 02 F/bee when compared to the control group throughout the oral testing period of 48 hours.

The 48-hour LD50 values are summarised in Table A 33.

**Contact test**

No mortality occurred in the control groups either treated with deionised water or tween solution after 48 hours. In the test item treatment, no mortality occurred after thoracic application of ≤ 1000 μg BAS 743 02 F/bee, after 48 hours.

The LD50 (48 h) was > 1000 μg BAS 743 02 F/bee that is corresponding to > 527.0 μg total a.s./bee.

The 48-hour LD50 values are summarised in Table A 33.

The 24-hour LD50 for the reference item was determined to be 0.153 μg a.s./bee (95% confidence limits 0.141 – 0.165 μg a.s./bee).

Table A 33: LD50 values of the oral and contact toxicity tests with BAS 743 02 F and the honeybee *Apis mellifera* L.

|  |  |  |
| --- | --- | --- |
|  | **Oral toxicity test** | **Contact toxicity test** |
| 48-hour LD50 (µg product/bee) | >844 | >1000 |
| 48-hour LD50 (µg total a.s./bee) | >444.7 | > 527.0 |

**D. VALIDITY CRITERIA**

All validity criteria were met.

Table A 34: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| **Oral toxicity test** | |
| In the control, mortality after 48 hours should be ≤10% | 0.0% |
| The 24-hour LD50 of the reference item should be between 0.10 – 0.35 µg a.s./bee | 0.105 µg a.s./bee |
| **Contact toxicity test** | |
| In the controls, mortality after 48 hours should be ≤10% | 0.0% in both controls |
| In the contact toxicity test, the 24-hour LD50 of the reference item should be between 0.10 – 0.30 µg a.s./bee | 0.153 µg a.s./bee |

**E. DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

The acute contact and oral toxicity of BAS 743 02 F was tested on honeybees under laboratory conditions over 48 hours.

In the contact toxicity test, no mortality occurred in the control groups either treated with deionised water or 1 % v/v tween solution. In the test item treatment, no mortality was observed after thoracic application of ≤ 1000 μg BAS 743 02 F/bee, after 48 hours. The LD50 (48 h) was > 1000 μg BAS 743 02 F/bee, corresponding to > 527.0 μg total a.s./bee.

In the oral toxicity test, no mortality occurred in the control group fed with pure 50 % w/v sucrose solution. In the test item treatment, statistically significant mortality of 20.0 % was observed after oral consumption of 816 μg BAS 743 02 F/bee, after 48 hours. The LD50 (48 h) was > 844 μg BAS 743 02 F/bee, corresponding to > 444.7 μg total a.s./bee.

Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 213 and 214 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.1.1.1/02 |
| Report | Acute toxicity of BAS 743 03 F to the honeybee *Apis mellifera* L. under laboratory conditions  Poráczki, K., 2023b  XXXX Study ID: 933750\_1  XXXX Doc ID: 2022/2033729 |
| Guideline(s): | OECD 213: Honeybees, Acute Oral Toxicity Test (1998), OECD 214: Honeybees, Acute Contact Toxicity Test (1998) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The acute contact and oral toxicity of BAS 743 03 F was tested on honeybees (*Apis mellifera* L.) under laboratory conditions over 48 hours. Nominal test doses were 62.5, 125, 250, 500 and 1000 µg product/bee in both tests, that is corresponding to 464.9, 232.5, 116.2, 58.1 and 29.1 μg total a.s./bee, respectively (based on sum of nominal content of a.s.). Control groups were run in parallel (50% (w/v) sucrose solution in the oral test and a water and a wetting agent control in the contact test). Every treatment group in both tests comprised 3 replicates, with 10 bees per replicate.

In the contact toxicity test, no mortality occurred in the control groups either treated with deionised water or 1 % v/v tween solution. In the test item treatment, no mortality was observed after thoracic application of ≤ 1000 μg BAS 743 03 F/bee, after 48 hours. The LD50 (48 h) was > 1000 μg BAS 743 03 F/bee, corresponding to > 464.9μg total a.s./bee.

In the oral toxicity test, no mortality occurred in the control group fed with pure 50 % w/v sucrose solution. In the test item treatment, statistically significant mortality of 20.0 % was observed after oral consumption of 816 μg BAS 743 03 F/bee, after 48 hours. The LD50 (48 h) was > 816 μg BAS 743 02 F/bee, corresponding to > 379.5 μg total a.s./bee.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 03 F |
| Description: | Liquid (colour not reported) |
| Lot/Batch: | FRE-002223 |
| Active substance content: | Ametoctradin (BAS 650 F), nominal 120.0 g/L, measured 120.2 g/L;  Propamocarb, nominal 378.0 g/L, measured 376.7 g/L |
| Density: | 1.079 g/cm³ (at 20°C) |
| 1. **Control:** | Oral test: 50% (w/v) sucrose solution  Contact test: Control 1: deionised water; Control 2: deionised water + 1% v/v wetting agent Tween 80 |
| 1. **Reference item:** | Dimethoate EC 400 (BAS 152 11 I) (401.7 g/L analysed) |
|  |  |

**B. STUDY DESIGN AND METHODS**

|  |  |
| --- | --- |
| 1. **Test species:** | Honeybee *Apis mellifera* L. Buckfast (Insecta, Hymenoptera, Apoidea) |
| **Age/life stage:** | Young adult female worker bees (3-5 weeks old) |
| **Source:** | BioChem agrar GmbH, Kupferstr. 6, 04827 Machern OT Gerichshain, Germany |
| **Diet:** | 50% w/v sucrose solution provided after application (immediately after thoracic application in the contact test and after removal of application feeders in the oral test). Food was provided *ad libitum*. |
| 1. **Test units:** | Disposable cardboard cages 95 mm x 50 mm x 65 mm (length x width x height) with holes in the bottom for ventilation and a glass plate at the front |
| 1. **Environmental conditions** |  |
| **Temperature:** | 24.2 – 24.4°C |
| **Relative humidity:** | 59.1 – 62.9% |
| **Photoperiod:** | 24-hour darkness (except for diffuse artificial light during assessments and handling) |

1. **Test organism and treatment:**

Both tests were performed as dose-response tests with 3 replicates per treatment group, each comprising one cage containing 10 bees.

1. **Oral toxicity test**

The bees were starved for 60 minutes before the test. At the start of the test, a quantity of 200 µL (corresponding to 0.2376 g) of the test item solution was offered to each cage, equivalent to 20 µL/bee. The test substance and toxic reference were administered in 50% w/v sucrose solution at the doses outlined in Table A 35.

Honeybees of a distinct group were assumed to consume approximately the same amount of the test item due to their social feeding behaviour. Bees in the control group were fed untreated 50% w/v sucrose solution.

Feeding tubes were weighed prior to addition of the sucrose solution. The feeders containing the appropriate diet were introduced through a hole in the roof of the cage. The feeding tubes were visibly empty approximately 3.5 hours after application. All feeding tubes were reweighed to determine the exact quantity of the test solution consumed and were then replaced with feeding tubes containing untreated 50% w/v sucrose solution.

Table A 35: Applied and consumed dosages in the oral toxicity test

| **Treatment group** | **Item applied** | **Applied dosages** | | **Actual uptake of the applied item** | |
| --- | --- | --- | --- | --- | --- |
| Control | Sucrose solution1 | -- | | -- | |
|  | | **(µg product/bee)** | **(µg total a.s./bee)** | **(µg product/bee)** | **(µg total a.s./bee)** |
| Test item | BAS 743 03 F\* | 62.5 | 29.1 | 62.2 | 28.9 |
| 125 | 58.1 | 124 | 57.7 |
| 250 | 116.2 | 250 | 116.1 |
| 500 | 232.5 | 496 | 230.9 |
| 1000 | 464.9 | 816 | 379.5 |
| Reference item | Dimethoate EC 400\*\* | 0.144 | 0.054 | 0.144 | 0.054 |
| 0.240 | 0.090 | 0.240 | 0.090 |
| 0.401 | 0.150 | 0.400 | 0.149 |
| 0.668 | 0.250 | 0.667 | 0.249 |

\* based on sum of nominal content of a.s.

\*\* based on analysed content of a.s.

1 respective solvent control for the test item and reference item treatment

Calculations are performed with non-rounded values

1. **Contact toxicity test**

Bees in the test cage were anaesthetised with CO2 for approximately 30 seconds and placed into a petri dish prior to application. A single 2 μL droplet of either the test item, control or toxic reference solution was placed on the dorsal bee thorax of each bee using an Eppendorf Micropipette. 1% Tween 80 solution was used as a vehicle to ensure a good penetration or adhesion of the droplet on the bee body. Tween solution is non-toxic to honeybees at the concentration used. Doses used for the test item and reference item are outlined in Table A 36.

Table A 36: Applied dosages in the contact toxicity test

| **Treatment group** | **Item applied** | **Dose rates** | |
| --- | --- | --- | --- |
| Control | Deionised water | -- | |
| Tween solution | **--** | |
|  | | **(µg product/bee)** | **(µg total a.s./bee)** |
| Test item | BAS 743 03 F\* | 62.5 | 32.9 |
| 125 | 65.9 |
| 250 | 131.7 |
| 500 | 263.5 |
| 1000 | 527.0 |
| Reference item | Dimethoate EC 400\*\* | 0.282 | 0.250 |
| 0.376 | 0.187 |
| 0.501 | 0.141 |
| 0.668 | 0.105 |

\* based on sum of nominal content of a.s.   
\*\* based on analysed content of a.s.

1 respective solvent control for the test item and reference item treatment

Calculations are performed with non-rounded values

1. **Dose preparation:**

**Oral test**

0.500 g BAS 743 03 F was weighed out and filled up to 10 mL with 50% w/v sucrose solution which gave a 5.000 % w/v stock solution. This was then diluted sequentially to produce the remaining 4 doses. The application volume was 200 µL/cage (equivalent to 20 µL per bee).

**Contact test**

5.000 g BAS 743 03 F was weighed out and filled up to 10 mL with 1% v/v Tween solution which gave a 50.000 % (w/v) stock solution. This was then diluted to produce the remaining 4 doses.

1. **Measurements and observations:**

In both tests, assessments of bee mortality and behavioural effects were made after 4, 24 and 48 hours. During the behavioural assessments, bees were classified as either healthy (normal), affected (impaired locomotion) or moribund. Other effects such as abnormal quantity/colour of excretion were also observed and recorded. All evaluations were made in comparison with the controls.

1. **Statistical analysis:**

For statistical evaluation of the mortality results the Fisher’s Binomial Test with Bonferroni-Holm correction or Step-down Cochran-Armitage Test procedure was used. The accepted significance level was p ≤0.05 (one-sided greater). The median lethal doses (LD50) along with the 95 % confidence limits were calculated with Probit analysis, using linear maximum likelihood regression for evaluation of the reference item, only.

The statistical calculations were performed with the computer program ToxRat Professional 3.3.0. (2018)

**II. RESULTS AND DISCUSSION**

**C. BIOLOGICAL EFFECTS**

**Oral test**

No mortality occurred in the control group fed with pure sucrose solution, after 48 hours. In the test item treatment, statistically significant mortality of 20.0 % was observed after oral consumption of 816 μg BAS 743 03 F/bee after 48 hours. Therefore, the LD50 (48 h) was > 816 μg consumed product/bee that is corresponding to > 379.5 μg total a.s./bee. No behavioural impairments were observed after oral application of ≤ 816 μg BAS 743 03 F/bee when compared to the control group throughout the oral testing period of 48 hours.

The 48-hour LD50 values are summarised in Table A 37.

**Contact test**

No mortality occurred in the control groups either treated with deionised water or tween solution after 48 hours. In the test item treatment, no mortality occurred after thoracic application of ≤ 1000 μg BAS 743 03 F/bee, after 48 hours.

The LD50 (48 h) was > 1000 μg BAS 743 03 F/bee that is corresponding to > 464.9 μg total a.s./bee.

The 48-hour LD50 values are summarised in.Table A 37.

The 24-hour LD50 for the reference item was determined to be 0.153 μg a.s./bee (95% confidence limits 0.141 – 0.165 μg a.s./bee).

Table A 37: LD50 values of the oral and contact toxicity tests with BAS 743 03 F and the honeybee *Apis mellifera* L.

|  |  |  |
| --- | --- | --- |
|  | **Oral toxicity test** | **Contact toxicity test** |
| 48-hour LD50 (µg product/bee) | >816 | >1000 |
| 48-hour LD50 (µg total a.s./bee) | >379.5 | > 464.9 |

**D. VALIDITY CRITERIA**

All validity criteria were met.

Table A 38: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| **Oral toxicity test** | |
| In the control, mortality after 48 hours should be ≤10% | 0.0% |
| The 24-hour LD50 of the reference item should be between 0.10 – 0.35 µg a.s./bee | 0.105 µg a.s./bee |
| **Contact toxicity test** | |
| In the controls, mortality after 48 hours should be ≤10% | 0.0% in both controls |
| In the contact toxicity test, the 24-hour LD50 of the reference item should be between 0.10 – 0.30 µg a.s./bee | 0.153 µg a.s./bee |

**E. DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

The acute contact and oral toxicity of BAS 743 03 F was tested on honeybees under laboratory conditions over 48 hours.

In the contact toxicity test, no mortality occurred in the control groups either treated with deionised water or 1 % v/v tween solution. In the test item treatment, no mortality was observed after thoracic application of ≤ 1000 μg BAS 743 03 F/bee, after 48 hours. The LD50 (48 h) was > 1000 μg BAS 743 03 F/bee, corresponding to > 464.9 μg total a.s./bee.

In the oral toxicity test, no mortality occurred in the control group fed with pure 50 % w/v sucrose solution. In the test item treatment, statistically significant mortality of 20.0 % was observed after oral consumption of 816 μg BAS 743 02 F/bee, after 48 hours. The LD50 (48 h) was > 816 μg BAS 743 02 F/bee, corresponding to > 379.5 μg total a.s./bee.

Study 3

XXXX have a Letter of Access allowing them to rely on this study

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | None |
| Report | Effects of propamocarb-HCL tech. (Acute Contact and Oral)  on Honey Bees (*Apis mellifera* L.) in the Laboratory  Schmitzer, S.., 2014  Project ID: 91721035 |
| Guideline(s): | OECD 213 and 214 (1998) |
| Deviations: | Compared to OECD 213 and 2014 (1998):  Humidity during the test exceeded the range of 50 – 70% (71%). This deviation is considered minor and has no influence on outcome and integrity of the study. |
| GLP: | Yes | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

Executive Summary

The purpose of this study was to determine the acute oral and contact toxicity of propamocarb-HCL tech. to the honeybee (*Apis mellifera* L*.*). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were assessed as well.

Under laboratory conditions a total of 10 worker bees per replicate (50 individuals per treatment group) were exposed to a single dose of 109.0 µg a.s. per bee for 48 hours by topical application (contact limit test). Separate batches of 10 worker bees per replicate and treatment were exposed to a single dose of 122.1 µg a.s./bee by feeding application (oral limit test; value based on the actual intake of the test item).

The contact test comprised a water control group (tap water with 0.5 % Adhäsit). The oral test comprised a water control group (50 % w/w sucrose solution (in tap water)). In both tests, a toxic reference item (dimethoate) was included.

In the contact toxicity test the LD50 value (48 h) of propamocarb-HCL tech was > 109.0 µg a.s./bee.

In the oral toxicity test the LD50 value (48 h) was > 122.1 µg a.s./bee.

The study fulfils all validity criteria of current Guidelines OECD 213 (1998) and OECD 214 (1998).

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| **Test item:** | Propamocarb-HCL tech. |
| Description: | Liquid, colourless |
| Lot/Batch: | EK1C000648 |
| Active substance content: | Propamocarb-hydrochloride (AE B066752): 70.1 % w/w (analytical) |
| Density: | 1.09 g/mL (20 °C) |
| Storage conditions: | From 10 °C to 30 °C, under dark and dry conditions |
| Stability (expiry date): | 29.11.2015 |
| **Control:** | Oral Test: 50 % w/v sucrose solution (500 g sucrose/L tap water)  Contact Test: Tap water with 0.5 % Adhäsit\* (applied after anesthetization with CO2) |
| **Reference item:** | Perfekthion EC (BAS 152 11 I), dimethoate: 400.0 g/L (nominal), 400.9 g/L (analytical) |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. **Test organism:** | *Apis mellifera* L |
| **Age:** | Adult worker bees |
| **Source:** | In-house culture, from disease-free and queen-right colonies |
| **Diet:** | 50% w/v sucrose solution (500 g/L) *ad libitum* |

|  |  |
| --- | --- |
| 1. **Test units:** | Stainless steel cages (10 cm x 8.5 cm x 5.5 cm) with holes in the bottom for ventilation and a glass plate in front, lined with filter paper |
| 1. **Environmental conditions** |  |
| **Temperature:** | 24 - 25 °C; short-term deviations (< 2 hours) are not reported |
| **Relative humidity:** | 51 - 72 %; short-term deviations (< 2 hours) are not reported |
| **Photoperiod:** | Darkness, except during observation |

1. **Test organism and treatment:**

Both tests were performed as limit tests with 5 replicates per treatment group, each containing 10 bees.

1. **Oral toxicity test**

The bees were starved for 15 minutes before the test. The test item and reference item were applied in 50 % w/v sucrose solution, in doses outlined in Table A 39. For the control pure 50 % w/v sucrose solution was offered to the bees.

The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake was 1 hour 40 minutes for the test item treatments). After a maximum of 1 hour 40 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The mean target dose levels (e.g. 100 μg a.s./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sucrose solutions differed slightly from the nominal 20 mg/bee and results are given based on the measured consumption.

Table A 39: Applied and consumed dosages in the oral toxicity test

| **Treatment group** | **Item applied** | **Applied dosages** | **Actual uptake of the applied item** |
| --- | --- | --- | --- |
| Control | Sucrose solution | -- | -- |
|  | | **(µg total a.s./bee)** | **(µg total a.s./bee)** |
| Test item | Propamocarb | 109.0 | 122.1 |
| Reference item | Dimethoate | 0.30 | 0.32 |
| 0.15 | 0.16 |
| 0.08 | 0.08 |
| 0.05 | 0.05 |

1. **Contact toxicity test**

Bees in the test cage were anaesthetised with CO2 for approximately 20 seconds prior to application. The test item was applied as one 5 μL droplet of propamocarb-HCL tech., dissolved in tap water with 0.5 % Adhäsit, placed on the dorsal bee thorax using a calibrated pipette. The reference was applied as one 5 μL droplet of dimethoate, dissolved in tap water with 0.5 % Adhäsit. For the control, one 5 μL droplet of tap water containing 0.5 % Adhäsit was used. A 5 μL droplet was chosen in deviation to the guideline recommendation of a 1 μL droplet, since a higher volume ensured a more reliable dispersion of the test item; higher volumes are suitable and no adverse effects on the outcome of the study are to be expected. Doses used for the test item and reference item are outlined in Table A 40.

Table A 40: Applied dosages in the contact toxicity test

| **Treatment group** | **Item applied** | **Applied dosages** |
| --- | --- | --- |
| Control | tap water with 0.5 % Adhäsit | -- |
|  | | **(µg total a.s./bee)** |
| Test item | Propamocarb | 109.0 |
| Reference item | Dimethoate | 0.30 |
| 0.20 |
| 0.15 |
| 0.10 |

1. **Dose preparation:**

**Oral test**

78 mg of the test item was dissolved in 10 g 50 % w/v sucrose solution (stock solution). 20 mg of stock solution contained 109.0 μg of the a.s. of propamocarb-HCL tech. The 109.0 μg a.s./bee dose level would have been obtained if 20 mg of the stock solution per bee was ingested. A dose of 122.1 μg a.s./bee was obtained, because the bees ingested between 22 and 23 mg treated food per bee.

**Contact test**

311 mg of the test item was dissolved in 10 mL carrier (= water containing 0.5 % Adhäsit) (stock solution). 5 μL of the stock solution contained 109.0 μg of the a.s. of propamocarb-HCL tech. The 109.0 μg a.i./bee dose was obtained when 5 μL of the stock solution was applied to each bee.

1. **Measurements and observations:**

In both tests, assessments of bee mortality and behavioural effects were made after 4, 24 and 48 hours. During the behavioural assessments, bees were classified as either healthy (normal), affected (impaired locomotion), cramps, vomiting, apathy (delayed reactions) or moribund. Other effects such as abnormal quantity/colour of excretion were also observed and recorded. All evaluations were made in comparison with the controls.

1. **Statistical analysis:**

Results obtained from the bees treated with the test item and the reference item were compared to those obtained from the control in both the contact and oral tests. The contact and oral LD50 values of the reference item were estimated with Probit Analysis. It was not necessary to correct the reference item mortality, since no control mortality occurred in either the contact or oral toxicity tests, respectively. The NOED of the test item was estimated using Fisher’s Exact Test (pairwise comparison, one-sided greater, α = 0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ToxRat Solutions GmbH.

II. Results and Discussion

**C. BIOLOGICAL EFFECTS**

**Oral test**

In the oral toxicity test, the maximum nominal test level of propamocarb-HCL tech. (i.e. 109.0 μg a.s./bee) corresponded to an actual intake of 122.1 μg a.s./bee. At this dose level and in the control group (50 % w/v sucrose solution) no mortality occurred after 48 hours, respectively. No test item induced behavioural abnormalities occurred. Since only no mortality occurred in the 122.1 μg a.s./bee group, the oral LD50 can be considered as > 122.1 μg a.s./bee. 48-hour LD50 values are summarised in Table A 41.

The 24-hour LD50 for the reference item was determined to be 0.15 μg a.s./bee (95% confidence limits 0.14 – 0.17μg a.s./bee) based on actual consumption. Results demonstrated the sensitivity of the test system.

**Contact test**

At the end of the contact toxicity test (48 hours after application), 2 % mortality occurred at 109.0 μg a.s./bee. There was no mortality in the control (water + 0.5 % Adhäsit). No test item induced behavioural abnormalities occurred. Since only 2.0 % mortality occurred in the 109.0 μg a.s./bee group, the contact LD50 can be considered as > 109.0 μg a.s./bee. 48-hour LD50 values are summarised in Table A 41.

The 24-hour LD50 for the reference item was determined to be 0.25μg a.s./bee (95% confidence limits 0.22 – 0.29 μg a.s./bee).

Table A 41: LD50 values of the oral and contact toxicity tests with Propamocarb and the honeybee *Apis mellifera* L.

|  |  |  |
| --- | --- | --- |
|  | **Oral toxicity test** | **Contact toxicity test** |
| 48-hour LD50 (µg total a.s./bee) | >122.1 | > 109.0 |

**D. VALIDITY CRITERIA**

All validity criteria were met (Table A 42).

Table A 42: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| **Oral toxicity test** | |
| In the control, mortality after 48 hours should be ≤10% | 0.0% |
| The 24-hour LD50 of the reference item should be between 0.10 – 0.35 µg a.s./bee | 0.17 µg a.s./bee |
| **Contact toxicity test** | |
| In the controls, mortality after 48 hours should be ≤10% | 0.0% |
| In the contact toxicity test, the 24-hour LD50 of the reference item should be between 0.10 – 0.30 µg a.s./bee | 0.29 µg a.s./bee |

**E. DEFICIENCIES**

There were no deficiencies.

**II. Conclusion**

The toxicity of propamocarb-HCL tech. was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD50 (48 h) was > 109.0 μg a.s./bee. The oral LD50 (48 h) was > 122.1 μg a.s./bee.

Study 4

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 246 and 247 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.1.1.1/04 |
| Report | Acute toxicity of BAS 743 02 F to the bumblebee *Bombus terrestris* L. under laboratory conditions  Amsel, K., 2023  XXXX Study ID: 933752\_18  XXXX Doc ID: 2022/2033711 |
| Guideline(s): | OECD Guidelines 246 (2017) and OECD 247 (2017) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability/Reliability: | Yes |

**Executive Summary**

The acute oral and contact toxicity of BAS 743 02 F to the bumblebee (*Bombus terrestris* L.) were assessed in a 48-hour laboratory study. Mortality of bumblebees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were assessed as well.

In the contact toxicity test, bumblebee adults were exposed by topical application to a single dose of 1000.0 µg product/bumblebee for 48 hours. In total, three treatment groups were set up: two control groups, one dose rate of the test item and one dose rate of the reference item with 50 replicates each (30 for the reference item) and one bumblebee per replicate, respectively. No mortality occurred in the control groups and in the item treatment group. No behavioural effects of bumblebees were observed at the tested dose rate. The 48- hour LD50 oral was determined to be > 1000.0 µg product/bumblebee and the NOED was ≥ 1000.0 µg product/bumblebee.

In the oral toxicity test, bumblebee adults were exposed by administration of the test item in feeding solution to five nominal doses of 500.0, 250.0, 125.0, 62.5, 31.3 µg product/bumblebee (oral uptake was 271.2, 203.5, 114.5, 59.2 and 29.1 µg product/bumblebee). In total, three treatment groups were set up: one control group, five dose rates of the test item and one dose rate of the reference item with 30 replicates each with one bumblebee per replicate, respectively. No mortality occurred in the control group (50% (w/v) sucrose solution) and in all doses of the test item treatment group. The 48-hour LD50 contact was determined to be > 271.2 µg product/bumblebee and the NOED was ≥ 271.2 µg consumed product/bumblebee.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Description:** | SC (suspension concentrate) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Density:** | 1.080 g/cm3 |
| **Storage conditions:** | At room temperature (+5°C to +35°C) |
| **Stability (expiry date):** | 31 January 2024 |
| 1. **Control:** | Oral test: 50% (w/v) sucrose solution;  Contact test: water control (deionised water) and solvent control (deionised water with a wetting agent (0.5% (v/v) TritonX) |
| 1. **Reference item:** | Dimethoate EC 400, analysed content of dimethoate 401.7 g/L |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. **Test organism:** | *Bombus terrestris* L |
| **Age:** | Young adult worker bumblebees |
| **Source:** | Biobest Belgium N.V. Ilse Velden, 18, 2260 Westerlo, Belgium  delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany |
| **Acclimatisation:** | Bees were placed in the environmental test chamber for preconditioning for about 21 hours before application of the treatments with a starving period of 4 hours in the oral toxicity test before application of the treatments. |
| **Diet:** | 50% w/v sucrose solution |
| 1. **Test units:** | Nicot cages (part of the Nicot queen bee rearing system) with a length of 7 cm and a diameter of 2 cm. |
| 1. **Environmental conditions** |  |
| **Temperature:** | 24.9 – 25.4°C |
| **Relative humidity:** | 59 – 66% |
| **Photoperiod:** | Constant darkness throughout the test (diffuse artificial light only during handling and assessments) |

1. **Treatment:**

The study was conducted at BioChem agrar (Labor für biologische und chemische Analytik GmbH, 04827 Machern OT Gerichshain, Germany) between 27.09.2022 and 29.09.2022.

Under laboratory conditions a total of 50 worker bees per replicate (30 individuals in the positive control) were exposed to a single dose of 1000.0 µg product per bumblebee for 48 hours by topical application (contact limit test). Separate batches of 30 replicates of one bumblebee each were exposed to five nominal doses of 500.0, 250.0, 125.0, 62.5, 31.3 µg product/bumblebee by feeding application (the actual dose based on the actual intake of the test item were 271.2, 203.5, 114.5, 59.2, 29.1 µg product/bumblebee, respectively).

The contact test comprised a water control group (deionised water) as well as a solvent control (deionised water with 0.5% (v/v) TritonX solution). The oral test comprised a water control group (50 % w/w sugar (syrup) solution (in tap water)). As a toxic reference dimethoate (Dimethoate EC 400, 400.0 g/L) was applied at an actual dose of 26.8 µg product/bee (referring to 10.0 µg a.s./bee) in the contact test and 4.0 µg product/bee (referring to 1.5µg a.s./bee) in the oral test.

Application in the contact test: The test item was applied as one 4-μL droplet of the test solution (BAS 743 02 F in an appropriate carrier (TritonX solution (0.5% v/v)), placed on the dorsal bee thorax using a Eppendorf Micropipette. For the controls, one 4-μL droplet of deionised water or 0.5% (v/v) TritonX solution was used. The reference item was applied in 4-μL deionised water. A 4-µL droplet was chosen in deviation to the guideline recommendation of a 2-µL droplet, since a higher volume ensured a more reliable dispersion of the test item. Bees were anaesthetized with CO2 for ca. 20 seconds until they were immobilized immediately before application.

Application in the oral test: The test item, reference item and controls were applied *ad libitum* in 50 % w/v sucrose solution. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 4 hours, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

1. **Dose preparation:**

A stock solution of the test item was prepared by dissolving 6.250 g BAS 743 02 F into 25 mL with 0.5% (v/v) TritonX solution for the contact test. For the oral test, 0.625 g BAS 743 02 F and filled up to 50 mL with 50% (w/v) sucrose solution. Each of the remaining test concentrations were prepared by dissolving an adequate volume of the previous higher test concentration with 50% w/v sucrose solution (25 ml).

1. **Measurements and observations:**

Biological observations:

Each group of bees was observed for moribund, mortality, lethargy and impaired coordination at 4, 24, and 48 hours post-treatment. Bees showing impaired locomotion were counted as affected.

Physicochemical measurements:

Air temperature and relative humidity were recorded continuously during the study.

Verification of exposure concentration:

For verification of the exposure concentration, the test item solutions of the highest and lowest test concentration as well as the respective control solutions were sampled directly after preparation. Samples were analysed using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection.

1. **Statistical analysis:**

No statistical analysis was necessary since no control and test item mortality occurred during the contact and oral toxicity test.

1. **Description of the analytical procedures**

Methodology was validated to quantify the amount of BAS 743 02 F via its active ingredients Propamocarb and Ametoctradin (BAS 650 F) present in recovery samples pre-pared in test item solutions resulting from acute toxicity tests with BAS 743 02 F on bumblebees. The determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection. All samples were extracted prior to sample analysis with 50/50 (v/v) (acetonitrile + 0.5% (v/v) formic acid))/water. The resulting extracts were further diluted into the range of the calibration curve before injecting into the HPLC-system. The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1 guideline. Five replicate dilutions were analyzed for each selected application solution. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 70 to 110%. Mean recoveries were 99.2% and 102% for Propamocarb and 101% and 105% for BAS 650 F for the fortification levels for the contact toxicity test as well as 97.3% and 97.8% for Propamocarb and 105% and 107% for BAS 650 F for the fortification levels for the oral toxicity test. The analyzed untreated control samples showed no residues at or above the LOD. The limit of quantitation was 1.45 mg/kg Propamocarb and 0.459 mg/kg BAS 650 F (contact toxicity test) and 1.52 mg/kg Propamocarb and 0.482 mg/kg BAS 650 F (oral toxicity test).

1. **Results and Discussion**
2. **biological effects**

No mortality occurred in the any of test item groups or controls after 48 hours in the oral and contact toxicity tests. No behavioural effects appeared during the test.

Table A 43: Mortality and behaviour of the bees in the contact toxicity test

| **Treatment group** | **Dosage** | **After 4 hours** | **After 24 hours** | **After 48 hours** |
| --- | --- | --- | --- | --- |
| **Mortality (%)** | **Mortality (%)** | **Mortality (%)** |
|
| Control | Water | 0.0 | 0.0 | 0.0 |
| 0.5% TritonX | 0.0 | 0.0 | 0.0 |
| BAS 743 02 F  [µg product/ bumblebee] | 1000.0 | 0.0 | 0.0 | 0.0 |
| Reference item  [µg a.s./ bumblebee] | 10.0 | 20.0 | 93.3 | 100.0 |

Dose levels of the test item are given in µg product/ bumblebee

Dose levels for the reference item given in μg a.s. (= dimethoate)/bee

Calculations are performed with non-rounded values

Table A 44: Mortality and behaviour of the bees in the oral toxicity test

| **Treatment group** | **Dosage (consumed)** | **After 4 hours** | **After 24 hours** | **After 48 hours** |
| --- | --- | --- | --- | --- |
| **Mortality (%)** | **Mortality (%)** | **Mortality (%)** |
|
| Control | Sucrose solution | 0.0 | 0.0 | 0.0 |
| BAS 743 02 F  [µg product/ bumblebee] | 271.2 | 0.0 | 0.0 | 0.0 |
| 203.5 | 0.0 | 0.0 | 0.0 |
| 114.5 | 0.0 | 0.0 | 0.0 |
| 59.2 | 0.0 | 0.0 | 0.0 |
| 29.1 | 0.0 | 0.0 | 0.0 |
| Reference item  [µg a.s./ bumblebee] | 1.46 | 13.3 | 93.3 | 100.0 |

Dose levels of the test item are given in µg product/ bumblebee

Dose levels for the reference item given in μg a.s. (= dimethoate)/bee

Calculations are performed with non-rounded values

1. **Analytical results**

Oral toxicity test solutions: The recoveries of BAS 743 02 F analyzed via its active ingredients Propamocarb and BAS 650 F in treated test item solutions were 98.1% Propamocarb and 103% BAS 650 F for the applied test concentration (AT) of the contact toxicity test as well as 102% Propamocarb and 113% BAS 650 F for the applied test concentration (ET) as well as 98.4% Propamocarb and 115% BAS 650 F for the applied test concentration (AT) of the oral toxicity test. No active substance was detected in the control sample.

Contact toxicity test solutions: the recoveries of BAS 743 02 F in test item solutions of the treatment were 98.1% Propamocarb and 103% Ametoctradin. No active substance was detected in the control sample

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 45: Validity criteria according to OECD 246 (2017) and OECD 247 (2017)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Validity criterion** | | | **Occurred** | **Recommended** |
| Control mortality (48 hours) | Contact test | Deionised water | 0.0% 0.0% | ≤ 10% |
| 0.5% (v/v) TritonX solution |
| Oral test | Sucrose solution | 0.0% | ≤ 10% |
| Mortality reference item (48 hours) | Contact toxicity test | | 100.0% | ≥ 50% |
| Oral toxicity test | | 100.0% | ≥ 50% |

1. **DEFICIENCIES**

There were no deviations with impact on quality and integrity of the study.

1. **Conclusion**

The acute contact and oral toxicity of BAS 743 02 F was tested on bumblebees under laboratory conditions for a period of 48 hours. The resulting 48-hour LD50 in the contact test was > 1000.0 µg product/bumblebee and the NOED was ≥ 1000.0 µg product/bumblebee. The resulting 48-hour LD50 in the oral test was > 271.2 µg consumed product/bumblebee and the NOED was ≥ 271.2 µg consumed product/bumblebee.

Study 5

XXXX have a Letter of Access allowing them to rely on this study

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Data point: | None |
| Report | Propamocarb-HCL tech.: Effects (Acute Oral) on Bumblebees (*Bombus terrestris* L.) in the Laboratory  Tänzler, V., 2015  Project No: 99901105 |
| Guideline(s): | van der Steen (2001) and OECD 213 (1998) with modifications and adaptions, Ring test bumblebee acute oral toxicity (ICPPR non-apis group, 2014)) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability/Reliability: | Yes |

**Executive Summary**

The acute oral toxicity of Propamocarb-HCL tech to the bumblebee (*Bombus terrestris* L.) were assessed in a 48h laboratory study. Mortality of bumblebees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were assessed as well.

Under laboratory conditions a total of 50 replicates of one worker bees each (50 individuals per treatment group) were exposed to a single dose of 250 µg product/bumblebee for 48 h by feeding application (oral limit test), while the actual dose based on the actual intake of the test item was 198.7 µg product/bumblebee. The oral test comprised a water control group (50 % w/w sugar (syrup) solution (in tap water)) as well as a solvent control group (50 % w/v sucrose solution containing 5 % acetone). A toxic reference item (dimethoate) was included.

In the oral toxicity test, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. No mortality was observed after oral consumption of 198.7 µg product/bumblebee within 48 hours. No behavioural effects of bumblebees were observed at the tested dose rate in the oral toxicity test. The 48 h LD50 was determined to be > 198.7µg a.s./bumblebee and the NOED was ≥ 198.7µg consumed a.s./bumblebee.

All validity criteria of the respective test guidelines were met.

**I MATERIALS AND METHODS**

1. **MATERIALS**

|  |  |
| --- | --- |
| **Test item:** | Propamocarb-HCL tech |
| **Description:** | Liquid, colourless |
| **Lot/Batch:** | PMC-2014-07-3 |
| **Active substance content:** | Propamocarb-HCL (AE B066752): 69.7 % w/w (analytical), according to certificate of analysis |
| **Density:** | Not reported |
| **Storage conditions:** | +10 to +30 °C, in the dark |
| **Stability (expiry date):** | 08 August 2016 |
| **Control:** | Water control 50% (w/v) sucrose solution;  Solvent control: 50 % w/v sucrose solution containing 5 % acetone |
| **Reference item:** | Dimethoate (BAS 152 11 I), analysed content of dimethoate 400.9 g/L |

**II study design and methods**

|  |  |
| --- | --- |
| 1. **Test organism:** | *Bombus terrestris* L |
| **Age:** | Adult worker bumblebees from healthy and queen-right colonies |
| **Source:** | Biobest Belgium N.V. Ilse Velden, 18, 2260 Westerlo, Belgium  delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany |
| **Acclimatisation:** | The bumblebees were acclimatised to the test conditions over night with ad libitum access to an untreated 50 % w/v sucrose solution. Acclimatisation period: 23 hours 30 minutes. |
| **Diet:** | 50 % w/v sucrose solution *ad libitum*; was given directly after treatment using syringes,  Starvation 220 to 270 minutes for all treatment groups in the oral test, prior to application |
| 1. **Test units:** | Cylindrical, latticed plastic cages with a length of approximately 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening. |
| 1. **Environmental conditions** |  |
| **Temperature:** | Acclimatisation: mean 25.1 °C, exposure: mean 25.1 °C |
| **Relative humidity:** | Acclimatisation: mean 57.1 %, exposure: mean 56.6 % |
| **Photoperiod:** | Constant darkness except during observations |

1. **Treatment:**

Under laboratory conditions a total of 50 worker bees per replicate were exposed to a single dose of 250.0 µg product per bumblebee for 48 hours by by feeding application (oral limit test; while the actual dose based on the actual intake of the test item were 198.7 µg product/bumblebee).

The oral test comprised a water control group (50 % w/w sugar (syrup) solution (in tap water)) as well as a solvent control group (50 % w/v sucrose solution containing 5 % acetone). As a toxic reference dimethoate (BAS 152 11 I, 400.0 g/L (nominal), 400.9g/L (analytical)) was applied at an actual dose of 4 µg a.s./bee.

Application in the oral test: The test item, reference item and controls were applied *ad libitum* in 50 % w/v sucrose solution. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 6 hours, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

1. **Dose preparation:**

For the test item treatment, Propamocarb-HCL tech. was diluted in 50 % w/v sucrose solution containing 5 % acetone. 712 mg of the test item were dissolved in 5 ml acetone (stock solution). 0.63 ml of the stock solution was added to 9.5 g of 50 % w/v sucrose solution in order to receive the final feeding solution. 40 mg of the final feeding solution contained 250 μg of the active ingredient. The 250 μg a.s./bumblebee dose level would be obtained if 40 mg of the final feeding solution per bumblebee were ingested. A mean dose of 198.7 μg a.s./bumblebee was obtained, because the uptake of final feeding solution was not exactly 40 mg per bumblebee.

1. **Measurements and observations:**

Biological observations:

After collection from the hive the bumblebees were kept in test units. Care was taken that bumblebee size and variation in size was as similar as technically possible in all treatment groups, by visual inspection. Each bumblebee was weighed individually after anesthetisation with CO2. Each group of bees was observed for moribund, mortality, lethargy and impaired coordination at 4, 24, and 48 hours post-treatment. Bees showing impaired locomotion were counted as affected.

Physicochemical measurements:

Air temperature and relative humidity were recorded with suitable instruments.

1. **Statistical analysis:**

Results obtained from the bumblebees treated with the test item and the reference item were compared to those obtained from the solvent control in the oral test. The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, α = 0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. Satistical analysis was performed using ToxRatPro 2.10.

**III Results and Discussion**

1. **biological effects**

No mortality occurred in the any of test item groups after 48 hours in the oral toxicity test. No mortality occurred in the controls.

No behavioural effects appeared during the test.

Table A 46 Mortality and behaviour of the bees in the oral toxicity test

| **Treatment group** | **Dosage (consumed)**  **(μg a.s./bumblebee)** | **After 4 hours** | | **After 24 hours** | | **After 48 hours** | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Mortality (%)** | **BA** | **Mortality (%)** | **BA** | **Mortality (%)** | **BA** |
| Control | Water | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 |
| solvent | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 |
| Propamocarb | 198.7 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 |
| Reference item | 2.4 | 0.0 | 20 | 60.0 | 0 | 60.0 | 0 |

BA Behavioural abnormalities

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 47 Validity criteria according to OECD 247 (2017)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Validity criterion** | | | **Occurred** | **Recommended** |
| Control mortality (48 hours) | Oral test | Water control | 0.0% | ≤ 10% |
| Solvent control | 0.0% | ≤ 10% |
| Mortality reference item (48 hours) | Oral toxicity test | | 60.0% | ≥ 50% |

1. **DEFICIENCIES**

There were no deviations with impact on quality and integrity of the study.

**IV Conclusion**

The acute oral toxicity of Propamocarb-HCL tech to the bumblebee (*Bombus terrestris* L.) were assessed in a 48h laboratory study. The oral NOED value was calculated to be ≥ 198.7 μg a.s./bumblebee. The oral LD50 value was > 198.7 μg a.s./bumblebee.

* + - * 1. KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to point KCP 10.3.1.1.1.

* + - 1. KCP 10.3.1.2. Chronic toxicity to bees
         1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.1.2/01 |
| Report | Chronic toxicity of BAS 650 00 F to the honeybee (*Apis mellifera* L.) under laboratory conditions  Ruhland S., 2015  Report No EU-141048049B  XXXX DocID 2014/1111114 |
| Guideline(s): | OECD 213 (1998), Decourty et al. (2005), Suchail et al. (2001), CEB No. 230 (2012), Current ring test protocol of the AG-Bienenschutz (2014) |
| Deviations: | No |
| GLP: | Yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany) |
| Acceptability: | Yes |

**Executive Summary**

In a 10-day chronic toxicity feeding test, 1-4 day old worker honeybees (*Apis mellifera carnica* L.) were exposed to BAS 650 00 F. The toxicity of the test item was determined at 2.7, 6.3, 12.1, 26.5 and 47.3 µg consumed a.s./bee/day (equivalent to nominal doses of 3.0, 6.0, 12.0, 23.9 and 47.9 µg a.s./bee/day), corresponding to concentrations of 0.077, 0.154, 0.307, 0.615 and 1.230 g a.s./kg food. Additionally, honeybees were treated with dimethoate as reference item or with an untreated control.

In the chronic toxicity feeding test, the control group showed a mean mortality of 8.3% after 10 days of testing. In the test item group, bees consuming doses of 12.1, 26.5 and 47.3 μg a.s./bee/day revealed mean mortalities of 73.3%, 98.3% and 100.0%, respectively, which were statistically significant increased compared to the control group after 10 days.

In the course of the test several bees were described as affected in terms of moving uncoordinated or moribund in the three highest test item dosages (12.1, 26.5, and 47.3 μg consumed a.s./bee/day). On the last day of the test, 8 out of 16 remaining bees were described as moribund in 12.1 μg consumed a.s./bee/day treatment while no behavioral abnormalities occurred in the two lowest test item dosages.

In a 10-day chronic toxicity feeding test with BAS 650 00 F, the LD50 and LC50 were determined to be 10.2 μg consumed a.s./bee/day and 0.254 g a.s./kg food, respectively. The NOED was 6.3 μg consumed a.s./bee/day and the NOEC was 0.154 g a.s./kg food.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. Test item: | BAS 650 00 F |
| Description: | Not reported |
| Lot/Batch: | FRE-001077 |
| Active substance content: | Ametoctradin (BAS 650 F, Reg. No. 4 993 353): 205.6 g/L analyzed (200.0 g/L nominal) |
| Density: | 1.042 g/cm3 at 20°C |
| 1. Control: | Control 1: untreated 50 % (w/v) aqueous sucrose solution |
| 1. Reference item: | dimethoate, 400 g/L nominal, 411.20 g/L analysed |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. Test species: | *Apis mellifera carnica* L. (honeybee), deriving from healthy and queen-right colonies |
| Age/life stage: | Maximum 1-4 days old at test start |
| Source: | Bienenfarm Kern GmbH, Leipzig, Germany. |
| Diet: | 50 % (w/v) aqueous sucrose solution, |
| 1. Test units: | Aluminium cages with the dimensions (95 mm width × 60 mm depth × 70 mm height) with holes in the lateral walls for ventilation and two glass plates (one in the front and one in the back) for observations |
| 1. Environmental conditions |  |
| Temperature: | 32.6 – 33.2°C |
| Relative humidity: | 57% - 62% |
| Photoperiod: | Constant darkness (artificial light of approx. 100 lux during assessments and exchange of feeders) |

1. **Test organism and treatment:**

In a 10-day test, young adults of *Apis mellifera* L. were daily exposed to 5 doses of BAS 650 00 F in treated food (50% (*w*/*v*) aqueous sucrose solution). In total, 3 treatment groups were set up: 5 doses of the test item, one untreated control group fed with 50% (*w/v*) aqueous sucrose solution and 4 doses of the reference item with 3 replicates per dose and 20 bees per replicate. Assessments of bee mortality and behavioral effects were done daily during the study. Nominal test concentrations were 2.7, 6.3, 12.1, 26.5 and 47.3 µg consumed a.s./bee/day (equivalent to nominal doses of 3.0, 6.0, 12.0, 23.9 and 47.9 µg a.s./bee/day), corresponding to concentrations of 0.077, 0.154, 0.307, 0.615 and 1.230 g a.s./kg food; reference item: 5.9, 9.8, 16.4 and 27.3 ng dimethoate/bee/day, corresponding to 0.152, 0.253, 0.421 and 0.702 mg a.s./kg food.

1. **Measurements and observations:**

Mortality and behavioural abnormalities were recorded

1. **Statistical analysis:**

Descriptive statistics; Fisher’s Exact Binomial test with Bonferroni Correction for mortality data (one-sided greater, α = 0.05); Probit analysis using linear maximum likelihood regression for calculation of LC50/LD50 of the test item.

**II. RESULTS AND DISCUSSION**

In the chronic toxicity feeding test, the control group showed a mean mortality of 8.3% after 10 days of testing. In the test item group, bees consuming doses of 12.1, 26.5 and 47.3 μg a.s./bee/day revealed mean mortalities of 73.3%, 98.3% and 100.0%, respectively, which were statistically significant increased compared to the control group after 10 days (Fisher’s Exact Binomial test with Bonferroni Correction, one-sided greater, α = 0.05). In the course of the test several bees were described as affected in terms of moving uncoordinated or moribund in the three highest test item dosages (12.1, 26.5, and 47.3 μg consumed a.s./bee/day). On the last day of the test 8 out of 16 remaining bees were described as moribund 12.1 μg consumed a.s./bee/day treatment while no behavioral abnormalities occurred in the two lowest test item dosages.

The results are summarized in Table A 48.

Table A 48: Effects of BAS 650 00 F to *Apis mellifera carnica* L*.* in a 10-day chronic toxicity feeding test

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | | | **Mortality after 10 days** | | |
| **Nominal dosage [µg a.s./bee/day]** | **Consumed dosage [µg a.s./bee/day]** | | **Concentration [g a.s./kg food]** | **Mean mortality [%]** | **Corrected mortality 2)** | **Mean other observations 1)** |
| Control | Control | | Control | 8.3 | -- | 0 out of 55 |
| 3.0 | 2.7 | | 0.077 | 1.7 | 0.0 | 0 out of 59 |
| 6.0 | 6.3 | | 0.154 | 16.7 | 9.1 | 0 out of 50 |
| 12.0 | 12.1 | | 0.307 | 73.3 \* | 70.9 | 8 out of 16 |
| 23.9 | 26.5 | | 0.615 | 98.3 \* | 98.2 | 0 out of 1 |
| 47.9 | 47.3 | | 1.230 | 100.0 \* | 99.9 | 0 out of 0 |
| **Endpoints [10 days]** | | | | | | |
| **Test item dose [µg consumed a.s./bee/day]** | | **LD50 3)** | | 10.2 (95% CL: 9.2 – 11.3) | | |
| **NOED** | | 6.3 | | |
| **Test item concentrations  [g a.s./kg food]** | | **LC50 \*3)** | | 0.254 (95% CL: 0.230-0.280) | | |
| **NOEC** | | 0.154 | | |

1) Number of bees with behavior abnormalities referring to the number of remaining bees.

2) Corrected mortality according Schneider-Orelli (1947).

3) Median lethal dose/concentration (and 95% confidence limits / lower-upper) was calculated by using Probit analysis (linear max. likelihood regression).

\* Statistically significant difference in comparison to the control (Fisher`s Exact Binomial Test with Bonferroni Correction; α = 0.05; one sided greater).

For the reference item no LD50/LC50 could be determined. The highest reference dosage tested in the study was 27.3 ng a.s./bee/day (actual consumption on average per day: 17.5 ng a.s./bee), which caused a mean mortality of 68.3%.

**C. Validity criteria**

All validity criteria were met (Table A 49).

Table A 49: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| In the control(s), mean mortality should be ≤15% after 10 days exposure | 8.3% |
| In the toxic reference test, mean mortality should be ≥50% after 10 days exposure | 68.3% |

**D. Deficiencies**

None

**III. CONCLUSION**

In a 10-day chronic toxicity feeding test with BAS 650 00 F, the LD50 and LC50 were determined to be 10.2 μg consumed a.s./bee/day and 0.254 g a.s./kg food, respectively. The NOED was 6.3 μg consumed a.s./bee/day and the NOEC was 0.154 g a.s./kg food.

* + - * 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 245 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.1.2/02 |
| Report | Chronic toxicity of BAS 743 02 F to the honey bee Apis mellifera L. under laboratory conditions  Ruhland S., 2023  Study number 933752-2  XXXX DocID 2022/2033709 |
| Guideline(s): | OECD 245 (2017) |
| Deviations: | No |
| GLP: | Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany) |
| Acceptability: | Yes |

**Executive Summary**

The chronic oral toxicity of BAS 743 02 F to 2-day old worker honeybees (*Apis mellifera* L. subspecies Buckfast) was determined in a 10-day toxicity feeding test. The toxicity of the test item was determined at 190, 95.5, 58.0, 41.7 and 15.9 µg consumed product/bee/day (equivalent to nominal doses of 800, 320, 128, 51.2 and 20.5 µg product/bee/day), corresponding to concentrations of 20.370, 8.148, 3.259, 1.304 and 0.521 g product/kg food. Additionally, honeybees were treated with dimethoate as reference item or with an untreated control and viscosifier control. Assessments of bee mortality, food consumption and behavioural abnormalities were conducted daily.

The blank control group showed a mean mortality of 3.3% after 10 days of testing while in the viscosifier control groups, a mean mortality of 0% was observed. In the test item group, bees consuming doses of 190, 95.5, 58.0, 41.7 and 15.9 µg product/bee/day revealed mean mortalities of 100.0, 66.7, 23.3, 3.3 and 0.0% respectively. Mortalities in the 190, 95.5 and 58.0 µg consumed product/bee/day treatment groups were statistically significantly increased compared to the viscosifier control group. There were no behavioural abnormalities in any of the treatment groups observed in the final assessment on the last day of the test.

The LDD50 was determined to be 78.6 µg consumed product/bee/day. The corresponding LC50 was determined to be 5.394 g product/kg food. The NOEDD was determined to be 41.7 µg consumed product/bee/day. The corresponding NOEC was determined to be 1.304 g product/kg food.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. Test item: | BAS 743 02 F |
| Description: | SC (suspension concentrate) |
| Lot/Batch: | FRE-002224 |
| Active substance content: | Propamocarb: nominal 432.0 g/L; analysed 431.0 g/L  Ametoctradin: nominal 137.14 g/L; analysed 137.7 g/L |
| Density: | 1.080 g/cm3 |
| 1. Control: | Blank control: untreated 50 % (w/v) aqueous sucrose solution  Viscosifier control: untreated 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan |
| 1. Reference item: | Dimethoate (Danadim Progress), 400 g/L nominal, 401.7 g/L analysed |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. Test species: | *Apis mellifera* L. subspecies Buckfast, deriving from healthy and queen-right colonies |
| Age/life stage: | Maximum 2 days old at test start |
| Source: | On-site apiary maintained by BioChem agrar GmbH, Kupferstraße 6, 04827 Machern OT Gerichshain, Germany |
| Diet: | 50 % (w/v) aqueous sucrose solution |
| 1. Test units: | Aluminium cages with the dimensions (95 mm width × 60 mm depth × 70 mm height) with holes in the lateral walls for ventilation and two glass plates (one in the front and one in the back) for observations |
| 1. Environmental conditions |  |
| Temperature: | 32.6 – 33.3°C |
| Relative humidity: | 53.3 – 68.8% |
| Photoperiod: | Constant darkness (diffuse artificial light only during assessments and exchange of feeders) |

1. **Test organism and treatment:**

In a 10-day chronic toxicity feeding test, max. 2-day old worker honeybees (*Apis mellifera* L. subspecies Buckfast) were exposed to BAS 743 02 F in treated food (50% (w/v) aqueous sucrose solution). In total, four treatment groups were set up: five doses of the test item, one untreated control group fed with 50% (w/v) aqueous sucrose solution, a viscosifier control group containing untreated 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan and one dose of the reference item with three replicates per dose and 10 bees per replicate. Assessments of bee mortality and behavioral effects were done daily during the study. Nominal test concentrations were 190, 95.5, 58.0, 41.7 and 15.9 µg consumed product/bee/day (equivalent to nominal doses of 800, 320, 128, 51.2 and 20.5 µg product/bee/day), corresponding to concentrations of 20.370, 8.148, 3.259, 1.304 and 0.521 g product/kg food. An additional group of honeybees was exposed to a daily application of dimethoate diluted in 50% (w/v) sucrose solution as a reference item at a nominal dose of 27.3 ng a.s./bee/day.

The treated/untreated food was provided *ad libitum* in a plastic syringe for about 24 hours (± 2 hours), then, old feeders were replaced by new feeders. The possible evaporation of feeding solutions from the feeders was investigated in additional test cages that contained no bees, only pre-weighed feeders containing diet of untreated control feeding solutions.

1. **Dose preparation:**

The test item feeding solutions were prepared freshly every day just before administration of food. A stock solution was prepared by mixing 1.212 g of BAS 743 02 F with 50 mL of 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan. Serial dilutions were then performed to prepare the remaining four stock solutions.

A stock solution of the reference item was prepared with 50 % w/v sucrose solution once and stored in the refrigerator. The reference item feeding solution was prepared at least every 4 days and stored in the refrigerator.

The 50% (w/v) sucrose solution and 50% (w/v) sucrose solution containing 0.1% (w/v) xanthan for the preparation of dilutions and feeding of the control groups was freshly prepared every day.

1. **Measurements and observations:**

Biological observations:

Mortality and behavioural abnormalities were recorded daily.

The syringes were replaced daily and food consumption was determined by weighing the syringes before being introduced into the cages and after they were replaced by new ones. The evaporation figure was then subtracted from the calculated uptake to give the real uptake accounting the loss by evaporation.

Physicochemical measurements:

Temperature and humidity were recorded continuously during the study.

Verification of exposure concentration:

For verification of the exposure concentration, the concentration of both active ingredients in the highest and lowest test item feeding solutions as well as in the respective control solutions applied on the first and last day of application (day 0 and day 9) were analysed using reversed phase high-performance liquid chromatography combined with mass spectrometry detection (RP-HPLC-MS/MS).

1. **Statistical analysis:**

If control mortality occurred, the corrected mortality was calculated for each concentration according to Abbott (1925)), modified by Schneider-Orelli (1947). Step-down Cochran-Armitage Test Procedure for mortality data and determination of NOEDD/NOEC (α = 0.05, one-sided greater) was used. LDDx and LCx values were calculated by Probit analysis using linear max. likelihood regression. The statistical software ToxRat Professional 3.3.0 (2018) was used.

1. **Description of the analytical procedures**

Methodology was validated to quantify the amount of BAS 743 02 F via its active ingredients Propamocarb and BAS 650 F present in recovery samples pre-pared in test item feeding solutions. The determination was conducted using reversed phase high performance liquid chromatography (RP-HPLC) with tandem mass spectrometric (MS/MS) detection. All samples were extracted prior to sample analysis with 50/50 (v/v) (acetonitrile + 0.5% (v/v) formic acid))/water. The resulting extracts were further diluted into the range of the calibration curve before injecting into the HPLC-system. The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1 guideline. The validation levels were 1.44 mg/kg (46% of lowest concentration in feeding solution) and 10926 mg/kg (134% of highest concentration in feeding solution) of Propamocarb as well as 0.459 mg/kg (46% of lowest concentration in feeding solution) and 3469 mg/kg (134% of highest concentration in feeding solution) of BAS 650 F corresponding to 3.61 mg/kg and 27315 mg/kg BAS 743 02 F. Five replicate dilutions were analyzed for each selected application solution. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 70 to 110%. The recoveries of BAS 743 02 F in feeding solutions were in the range of 97.3% to 104% for Propamocarb and 92.6% to 104% for BAS 650 F. The analyzed untreated control samples showed no residues at or above the LOD. The limit of quantitation was 1.44 mg/kg for Propamocarb and 0.459 mg/kg for BAS 650 F. The limit of detection was 0.311 mg/kg for Propamocarb and 0.0988 mg/kg for BAS 650 F.

**II. RESULTS AND DISCUSSION**

1. **biological effects**

After 10 days of continuous exposure, a mean mortality of 3.3% and 0% was observed in the blank control and viscosifier control groups, respectively. In the reference item group, a mean mortality of 76.7% was recorded. Taking into account the actual food uptake and evaporated amount of feeding solution, the bees in the test item group effectively consumed doses of 190, 95.5, 58.0, 41.7 and 15.9 µg product/bee/day which resulted in mortalities of 100.0, 66.7, 23.3, 3.3 and 0.0% after 10 days, respectively. Mortalities in the 190, 95.5 and 58.0 µg consumed product/bee/day treatment groups were statistically significantly increased compared to the viscosifier control group. The results are summarized in Table A 50.

Table A 50: Effects of BAS 743 02 F to *Apis mellifera* L*.* in a 10-day toxicity feeding test

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | | | **Mortality after 10 days** | | |
| **Nominal dosage [µg product/ bee/day]** | **Consumed dosage 1) [µg product/ bee/day]** | | **Concentration [g product/kg food]** | **Mean mortality [%]** | **Corrected mortality[%]** | **Mean other observations 2)** |
| Blank control | Control | | Control | 3.3 | -- | 0 out of 29 |
| Viscosifier control | Control | | Control | 0.0 | -- | 0 out of 30 |
| 20.5 | 15.9 | | 0.521 | 0.0 | -- | 0 out of 30 |
| 51.2 | 41.7 | | 1.304 | 3.3 | -- | 0 out of 29 |
| 128 | 58.0 | | 3.259 | 23.3\* | -- | 0 out of 23 |
| 320 | 95.5 | | 8.148 | 66.7\* | -- | 0 out of 10 |
| 800 | 190 | | 20.370 | 100.0\* | -- | -- |
| **Endpoints [10 days]** | | | | | | |
| **Test item dose 1) [µg consumed a.s./bee/day]** | | LDD50 3) | | 78.6 (70.2 – 89.6) | | |
| LDD20 3) | | 57.9 (49.2 – 65.1) | | |
| LDD10 3) | | 49.3 (39.8 – 56.5) | | |
| NOEDD 4) | | 41.7 | | |
| **Test item concentrations  [g a.s./kg food]** | | LC50 3) | | 5.394 (4.327 – 6.738) | | |
| LC20 3) | | 3.018 (2.163 – 3.809) | | |
| LC10 3) | | 2.228 (1.455 – 2.925) | | |
| NOEC 4) | | 1.304 | | |

1) Taking into account the actual food uptake and evaporation

2) Number of bees showing behavioural abnormalities referring to the number of remaining bees

3) Lethal dietary doses/concentrations (95%-cl lower/upper) were calculated by Probit analysis using linear max. likelihood regression

4) No observed effect dietary dose/concentration were determined using Step-down Cochran-Armitage Test Procedure (α = 0.05, one-sided greater)

\* Statistically significant difference in pairwise comparison between treatment and untreated viscosifier control (Step-down Cochran-Armitage Test Procedure; α = 0.05; one-sided greater)

In the test item treatment group, the overall mean daily food consumption ranged between 9.32 and 32.0 mg feeding solution/bee/day which corresponds to 23.7% and 81.5% of the expected daily amount. In blank control group, the bees consumed on average 36.1 mg feeding solution/bee/day (corresponding to 92.0% of the expected daily amount). In the viscosifier control group, the bees consumed on average 37.0 mg feeding solution/bee/day (corresponding to 94.2% of the expected daily amount).

For the reference item group (nominal dose of 27.3 ng a.s./bee/day, equivalent to an actual consumption of 14.4 ng a.s./bee), a mean mortality of 76.7% was recorded.

1. **ANALyTICAL RESULST**

The recovery rates of the active ingredients in the analysed samples of the test item feeding solutions were between ± 20% of the nominal concentrations. Therefore, the concentrations of active ingredients in the applied test item feeding solutions were verified and endpoints have been based on nominal concentrations. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ (limit of quantification). Since the samples of the test item feeding solutions were analysed within 30 days, storage stability of the active ingredients in sample matrix (50% (w/v) sucrose solution containing 0.1% (w/v) xanthan) was not addressed within the framework of the analytical phase of the study.

Table A 51: Analytical results

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Propamocarb** | | | **Ametoctradin** | | |
| **Nominal**  **Concentration**  **[mg/kg]** | **Residue**  **Concentration [mg/kg]** | **Mean Recovery**  **[%]** | **Nominal**  **Concentration**  **[mg/kg]** | **Residue**  **Concentration [mg/kg]** | **Mean Recovery**  **[%]** |
| 0.000 | <LOD | - | 0.000 | <LOD | - |
| 0.000 | <LOD | - | 0.000 | <LOD | - |
| 209 | 217 | 104 | 66.2 | 66.3 | 100 |
| 209 | 203 | 97.3 | 66.2 | 61.3 | 92.6 |
| 8148 | 8436 | 104 | 2587 | 2681 | 104 |
| 8148 | 8078 | 99.1 | 2587 | 2648 | 102 |

**C. Validity criteria**

All validity criteria were met (Table A 52).

Table A 52: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| In the control(s), mean mortality should be ≤15% after 10 days exposure | 3.3% (blank control)  0.0% (viscosifier control) |
| In the toxic reference test, mean mortality should be ≥50% after 10 days exposure | 76.7% |

**D. Deficiencies**

None

**III. CONCLUSION**

The chronic oral toxicity of BAS 743 02 F to young adult honeybees (*Apis mellifera* L.) was investigated in a 10-day chronic dose-response feeding study under laboratory conditions. The LDD50 was determined to be 78.6 µg consumed product/bee/day. The corresponding LC50 was determined to be 5.394 g product/kg food. The NOEDD was determined to be 41.7 µg consumed product/bee/day. The corresponding NOEC was determined to be 1.304 g product/kg food.

* + - * 1. Study 3

XXXX have a Letter of Access allowing them to rely on this study

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Data point: | None |
| Report | Propamocarb-HCL SL 722 - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Days Chronic Feeding Test under Laboratory Conditions  Pfeiffer, S., 2015  Study number S14-00178 |
| Guideline(s): | no specific guideline available. Based on OECD Guideline No. 213 (1998), CEB No. 230 (2012) and OECD Guideline Proposal (2013) |
| Deviations: | No |
| GLP: | Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany) |
| Acceptability: | Yes |

**Executive Summary**

In a 10-day chronic toxicity feeding test, max. 4-day old worker honeybees (*Apis mellifera* L.) were exposed to Propamocarb-HCL SL 722. The toxicity of the test item was determined at 187.5, 375, 750, 1500 and 3000 mg propamocarb-hydrochloride/kg feeding solution corresponding to test concentrations of 7.13, 13.6, 27.7, 52.9 and 85.7 µg a.s./bee/day. Additionally, honeybees were treated with dimethoate as reference item or with an untreated control.

In the chronic toxicity feeding test, the control group showed a mean mortality of 2.5% after 10 days of testing. The cumulative mortality at the concentration levels of 187.5, 375, 750, 1500 and 3000 mg product/kg feeding solution was 2.5, 0.0, 5.0, 0.0 and 7.5 %, respectively (corrected: 0.0, -2.6, 2.6, -2.6 and 5.1 %, respectively) at the final assessment. No significant differences were found.

In the control group and in the test item treatment group at the concentration levels of 375, 750 and 1500 mg product/kg feeding solution, no sub-lethal effects could be observed. At the concentration levels of 187.5 and 3000 mg product/kg feeding solution only one and two affected bees, respectively could be observed. The overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) at the concentration levels of 187.5, 375, 750 and 1500 mg product/kg feeding solution was not statistically significantly different (lower) when compared to the untreated control group (38.0, 36.2, 36.9, and 35.3 mg/bee/day at 187.5, 375, 750, and 1500 mg product/kg feeding solution, respectively compared to 39.4 mg/bee/day in the control group. At the concentration level of 3000 mg product/kg feeding solution, the overall mean daily consumption of feeding solution was statistically significantly lower when compared to the untreated control group (28.6 mg/bee/day at 3000 mg product/kg feeding solution compared to 39.4 mg/bee/day in the control group).

The NOEC for mortality after 10 days of continuous exposure was determined to be 3000 mg product/kg feeding solution. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 85.68 μg a.s./bee/day.

The LC50 after 10 days of continuous exposure was determined to be > 3000 mg product/kg feeding solution. The corresponding LDD50, based on the actual consumption of the respective feeding solutions, was determined to be >85.68 μg a.s./bee/day.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | Propamocarb-hydrochloride SL 722 |
| Description: | liquid / colourless |
| Lot/Batch: | 2014-002016-01 |
| Active substance content: | propamocarb-hydrochloride, nominal 722 g/L; analysed 716.0 g/L (66.2 % w/w) |
| Density: | 1.082 g/cm3 |
| 1. **Control:** | untreated 50 % (w/v) aqueous sucrose solution |
| 1. **Reference item:** | dimethoate (Perfekthion / BAS 152), 400 g/L nominal, 400.9 g/L analysed |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | *Apis mellifera* (honeybee), deriving from healthy and queen-right colonies |
| Age/life stage: | Maximum 4 days old at test start |
| Source: | breeding line of a beekeeper in Tiefenbronn, Germany (Klaus Hampel, Mühlhausenerstr. 1/1, 75233 Tiefenbronn, Germany). |
| Diet: | 50 % (w/v) aqueous sucrose solution |
| 1. **Test units:** | Stainless steel cages (base: 8 cm x 4 cm; height: 6 cm), front side of the cages were equipped with a transparent pane for observation, bottom perforated board for air supply, lined with filter paper. |
| 1. **Environmental conditions** |  |
| Temperature: | 32.2 – 33.8°C |
| Relative humidity: | 54.0 – 62.7 % |
| Photoperiod: | Constant darkness except during assessments |

1. **Test organism and treatment:**

In a 10-day chronic toxicity feeding test, max. 4-day old worker honeybees (*Apis mellifera)* were exposed to Propamocarb-hydrochloride SL 722 in treated food (50% (w/v) aqueous sucrose solution). In total, 3 treatment groups were set up: 5 doses of the test item, one untreated control group fed with 50% (w/v) aqueous sucrose solution and 1 dose of the reference item with 4 replicates per dose and 10 bees per replicate. Assessments of bee mortality and behavioral effects were done daily during the study. Test concentrations were 7.13, 13.6, 27.7, 52.9 and 85.7 µg a.s./bee/day corresponding to concentrations of 187.5, 375, 750, 1500 and 3000 mg product/kg food. An additional group of honeybees was exposed to a daily application of dimethoate diluted in 50% (w/v) sucrose solution as a reference item at a nominal dose of 0.90 mg dimethoate/kg feeding solution.

1. **Measurements and observations:**

Mortality and behavioural abnormalities were recorded daily.

1. **Analytical dose verification:**

Samples of the solutions prepared freshly every day throughout the 10 days continuous feeding period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the active substance of test item. Analytical samples of the feeding solutions of the control group and the test item groups were taken directly after the daily preparation of the feeding solutions. All samples were stored deep frozen (typically at ≤ - 18°C) immediately after sampling and maintained in a deep frozen condition and adequately separated during storage and shipment for subsequent chemical analysis. Analysis of the test item was conducted using Bayer CropScience method 01163 by means of reversed phase HPLC coupled with electrospray and mass spectrometry (MS/MS) detection.

1. **Statistical analysis:**

Fisher’s Exact Test (Bonferroni-Holms corrected, right-sided, p ≤ 0.05) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment group and to determine the NOEC and NOEDD based on mortality.

For the statistical comparison of the food consumption, non-rounded mean values per replicate over the entire test period were taken. Data of food consumption were statistically analysed by using the Williams t-test (left-sided, α = 0.05) depending on the results of the pre-test of Shapiro Wilks and F-Test (α = 0.05).

Statistical calculations were made by using the statistical program TOXRAT Professional 2.10.

**II. RESULTS AND DISCUSSION**

**Analytical results**

Mean recoveries of the test item in the tested solutions were in the range of 107 to 116% of nominal values. Since the recoveries were in the range of 70 – 120% of nominal, results are based on the nominal values.

Results are summarized in Table A 53.

Table A 53: Summary of actual test item concentrations in the feeding solutions of the control and test item treatment group

|  |  |  |  |
| --- | --- | --- | --- |
| **Nominal dosage [mg a.s./kg]** | **Mean actual concentration**  **[mg a.s./kg]** | **Mean calculated concentration**  **[mg product/kg]** | **Mean Recovery [%]** |
| Control | <LOD | <LOD | - |
| 187.5 | 182 | 217 | 116 |
| 375 | 336 | 400 | 107 |
| 750 | 683 | 815 | 109 |
| 1500 | 1357 | 1620 | 108 |
| 3000 | 2748 | 3280 | 109 |

LOQ = Limit of Quantification = 0.01 mg/kg for propamocarb HCL

LOD = Limit of Detection = 0.003 mg/kg for propamocarb HCL

**Biological results**

After 10 days of continuous exposure, a mean mortality of 2.5% was observed in the control group. In the reference item group, a mean mortality of 65.0 % (corrected 64.1 %) was recorded.

Taking into account the actual food uptake and evaporated amount of feeding solution, the bees in the test item group effectively consumed doses of 7.13, 13.6, 27.7, 52.9 and 85.7 µg a.s./bee/day which resulted in mortalities of 2.5, 0.0, 5.0, 0.0 and 7.5% after 10 days, respectively. (corrected: 0.0, -2.6, 2.6, -2.6 and 5.1 %, respectively). A LDD50 of > 85.68 μg a.s./bee/day and a NOEDD of 85.68 μg a.s./bee/day could be determined. In the control group and in the test item treatment group at the concentration levels of 375, 750 and 1500 mg poduct/kg feeding solution, no su-lethal effects could be observed. At the concentration levels of 187.5 and 3000 mg product/kg feeding solution only one and two affected bees, respectively could be observed.

The results are summarized in Table A 54.

Table A 54: Effects of Propamocarb-hydrochloride SL 722 to *Apis mellifera* L*.* in a 10-day chronic toxicity feeding test

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | | | **Mortality after 10 days** | | |
| **Nominal dosage [mg a.s./kg]** | **Dietary Dose2) [µg a.s./ bee/day]** | | **Overall mean consumption of feeding solution [mg/bee/day]** | **Mean mortality [%]** | **Corrected mortality1)** | **Mean other observations** |
| Control | Control | | 39.4 | 2.5 | -- | 0 |
| 187.5 | 7.13 | | 38.0 | 2.5 | 0.0 | 1 |
| 375 | 13.6 | | 36.2 | 0.0 | -2.6 | 0 |
| 750 | 27.7 | | 36.9 | 5.0 | 2.6 | 0 |
| 1500 | 52.9 | | 35.3 | 0.0 | -2.6 | 0 |
| 3000 | 85.7 | | 28.6\* | 7.5 | 5.1 | 2 |
| **Endpoints [10 days]** | | | | | | |
| **Test item dose  [µg consumed a.s./bee/day]** | | LDD50 2 | | > 85.68 | | |
| NOEDD 3 | | 85.68 | | |
| **Test item concentrations  [g a.s./kg food]** | | LC50 2 | | > 3000 | | |
| NOEC 3 | | 3000 | | |

1) Corrected morality according to SCHNEIDER-ORELLI (1947)

2) Dietary Dose (DD): mean uptake of test item (calculation based on the replicate values)

\* statistically significantly different compared to the control; Williams t-test (α = 0.05)

For the reference item no LD50/LC50 could be determined. The highest reference dosage tested in the study was 0.90 mg dimethoate/kg feeding solution (dietary dose of 0.03 μg a.s./bee/day), which caused a mean mortality of 65.0 %.

**C. Validity criteria**

All validity criteria of OECD TG 245 were met (Table A 55).

Table A 55: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| In the control(s), mean mortality should be ≤15% after 10 days exposure | 2.5% |
| In the toxic reference test, mean mortality should be ≥50% after 10 days exposure | 65.0% |

**D. Deficiencies**

Honey bees were up to 4 days old at test start. The study was conducted before the publication of the current guidance document and this deviation is considered minor and did not influence the integrity and outcome of the study.

**III. CONCLUSION**

The chronic oral toxicity of Propamocarb-hydrochloride SL 722 to young adult honeybees (*Apis mellifera*) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions. The LDD50 was determined to be > 85.68 µg consumed a.s./bee/day. The corresponding LC50 was determined to be > 3000 a.s. product/kg food. The NOEDD was determined to be 85.68 µg consumed a.s./bee/day. The corresponding NOEC was determined to be 3000 g a.s./kg food.

* + - 1. KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages
         1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.1.3/01 |
| Report | Repeated exposure of BAS 650 00 F to honey bee *(Apis mellifera*) larvae under laboratory conditions (*in vitro*)  Kleebaum K., 2016  Report No 14 10 48 061 B  XXXX DocID: 2014/1111115 |
| Guideline(s): | OECD 237 (2013) & OECD DRAFT Guidance Document for testing chemicals: Honey bee (*Apis mellifera*) larval toxicity test, repeated exposure (February 2014) |
| Deviations: | None |
| GLP: | Yes, (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany) |
| Acceptability: | Yes |

**Executive summary**

In a repeated exposure feeding toxicity test, first instar larvae (L1) of honeybees (*Apis mellifera* L., ssp. *Buckfast*) were repeatedly exposed to BAS 650 00 F diluted in the larvae food on four consecutive days (D3 to D6 after grafting). The toxicity of the test item was determined at total doses of 37.1, 92.6, 231.6, 579.0 and 1447.6 µg product/larva (corresponding to 7.1, 17.8, 44.4, 111.0, and 277.6 µg a.s./larva). The concentrations of the test item in the diet were 241, 602, 1504, 3760 and 9400 mg product/kg food (corresponding to 46, 115, 288, 721 and 1802 mg a.s./kg food). Additionally, honey bee larvae were treated with Dimethoate tech. as reference item. Untreated diet served as a control. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment. Additionally, qualitative observations were assessed after 96 and 120 hours. Pupal mortality was assessed at D15, and emergence of adults was evaluated at D22.

After 120 hours of oral exposure, a larval mortality of 8.3% was observed in the control. Pupal mortality between D8 and D22 was 12.1%. Thus, the control group showed a total mortality of 19.4% at D22. In the test item group, larval mortalities at D8 ranged between 0.0% and 25.0%. Pupal mortalities ranged between 31.4% and 90.3% in the same treatment groups. Thus, total mortalities at D22 ranged between 33.3% and 91.7%. None of the recorded mortality rates were statistically significantly different compared to the control.

At D8, larvae of all treatments, as well as larvae of control and reference item, had completely consumed their offered food. No additional abnormalities regarding body size were observed.

In the final assessment at D22, an adult emergence rate of 80.6% was determined for the honeybees in the control group. Adult honeybees emerged at rates of 66.7%, 38.9%, 25.0%, 16.7% and 8.3% following an application of 7.1, 17.8, 44.4, 111.0 and 277.6 µg a.s./larva, respectively, during the larval stages. Hence, statistically significant effects on adult emergence occurred at the four highest test item doses (17.8, 44.4, 111.0 and 277.6 µg a.s./larva, respectively).

In a repeated exposure larval toxicity study with BAS 650 00 F, the LD50 (larval mortality on D8) was determined to be > 1447.6 µg BAS 650 00 F/larva (> 277.6 µg a.s./larva), which is equivalent to an LC50 of > 9400 mg BAS 650 00 F/kg food (> 1802 mg a.s./kg food). The respective NOED was ≥ 1447.6 µg BAS 650 00 F/larva (≥ 277.6 µg a.s./larva) and the corresponding NOEC was ≥ 9400 mg BAS 650 00 F/kg food (≥ 1802 mg a.s./kg food).

In a repeated exposure larval toxicity study with BAS 650 00 F, the ED50 (successful adult emergence up to D22) was determined to be 120.3 µg BAS 650 00 F/larva (23.1 µg a.s./larva), which is equivalent to an EC50 of 781 mg BAS 650 00 F/kg food (149 mg a.s./kg food). The respective NOED was 37.1 µg BAS 650 00 F/larva (7.1 µg a.s./larva) and the corresponding NOEC was 241 mg BAS 650 00 F/kg food (46 mg a.s./kg food).

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 650 00 F |
| Active substance content: | Ametoctradin (BAS 650 F, Reg. No. 4 993 353): 202.5 g/L analyzed (200 g/L nominal) |
| Description: | Not reported |
| Lot/batch: | FRE-001643 |
| Density: | 1.043 g/cm3 |
|  |  |
| 1. **Control:** | Control: untreated diet |
|  |  |
| 1. **Reference item:** | Dimethoate tech. (analysed purity: 98.8 ± 0.5%) |

1. **STUDY DESIGN AND METHODS**

|  |  |
| --- | --- |
| 1. **Test species:** | Honeybee (*Apis mellifera* L.) |
| Age: | First instar larvae |
| Source: | In-house colony |
| Diet: | 50% aqueous yeast/sugar solution and 50% royal jelly |
|  |  |
| 1. **Environmental conditions** |  |
| Temperature: | 34 - 35°C |
| Relative humidity: | D1 - D8: 90 - 97%  D8 – D15: 78 - 82%  D15 – D22: 49% - 52% |
| Photoperiod: | Darkness (except during observations) |

1. **Test organism and treatment:**

Honeybee (*Apis mellifera* L.) L1 larva were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment. Larvae were repeatedly exposed to BAS 650 00 F diluted in the larval food (aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w)) on four consecutive days (D3 to D6 after grafting). After the applications, no additional feeding of the larvae took place.

In total, 7 treatment groups, each comprising 12 larvae per replicate, were set up: 5 doses of the test item, 1 control group (untreated control group (AC)), and 1 dose of the reference item with 3 replicates per dose, respectively.

Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, and D8). Additionally, other observations, such as small body size or large quantities of remaining food after 96 and 120 hours (on D7 and D8) were noted. Pupal mortality was assessed at D15, and emergence of adults was evaluated at D22.

In an analytical phase of the study, the concentration of the active substance in the base stock solution, sampled at D3, was determined.

1. **Test dose:**

Control: untreated diet (50% aqueous sugar solution with 50% royal jelly (w/w)).

Test item treatments:

|  |  |  |  |
| --- | --- | --- | --- |
| **Nominal doses** | | **Nominal concentrations** | |
| **[µg BAS 650 00 F/larva]** | **[µg a.s./larva]** | **[mg BAS 650 00 F/kg food]** | **[mg a.s./kg food]** |
| 37.1 | 7.1 | 241 | 46 |
| 92.6 | 17.8 | 602 | 115 |
| 231.6 | 44.4 | 1504 | 288 |
| 579.0 | 111.0 | 3760 | 721 |
| 1447.6 | 277.6 | 9400 | 1802 |

Reference item: treated diet with a dose of 6.2 µg dimethoate/larva (corresponding concentration: 40 mg a.s./kg food).

1. **Measurements and observations:**

Successful adult emergence (dose-effect relationship), mortality, qualitative observations: body size, remaining food.

1. **Statistical analysis:**

Descriptive statistics: Step-down Cochran-Armitage Test for mortality data and adult emergence on D22 or Chi² 2x2 Table Test with Bonferroni Correction for mortality data on D8 (both one-sided greater, α = 0.05) for determination of NOED/NOEC; Probit analysis (linear max. likelihood regression) for determination of ED50/EC50.

1. **RESULTS AND DISCUSSION**

After 120 hours of oral exposure, a larval mortality of 8.3% was observed in the control. Pupal mortality (between D8 and D22) was 12.1%. Thus, the control group showed a total mortality of 19.4% at D22. In the test item group, larval mortalities at D8 ranged between 0.0% and 25.0%. Pupal mortalities ranged between 31.4% and 90.3% in the same treatment groups. Thus, total mortalities at D22 ranged between 33.3% and 91.7%. None of the recorded mortality rates were statistically significantly different compared to the control (Step-down Cochran-Armitage Test for mortality data on D22 or Chi² 2x2 Table Test with Bonferroni Correction for mortality data on D8, both one-sided greater, α = 0.05).

At D8, larvae of all treatments, as well as larvae of control and reference item, had completely consumed their offered food. No additional abnormalities regarding body size were observed.

In the final assessment at D22, an adult emergence rate of 80.6% was determined for the honeybees in the control group. Adult honeybees emerged at rates of 66.7%, 38.9%, 25.0%, 16.7% and 8.3% following an application of 7.1, 17.8, 44.4, 111.0 and 277.6 µg a.s./larva, respectively, during the larval stages. Hence, statistically significant effects on adult emergence occurred at the four highest test item doses of 17.8, 44.4, 111.0 and 277.6 µg a.s./larva, respectively (Step-down Cochran-Armitage Test, one-sided greater, α = 0.05). The results are summarized in Table A 56 below.

Table A 56: Effects of BAS 650 00 F to *Apis mellifera* L*.* in a repeated exposure larval toxicity test

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Dose [µg /larva]** | **Concentration [mg/kg food]** | **Mean larval mortality D3 – D8 [%]** | | **Mean OO [%]** | **Mean pupal mortality D8 – D22 [%] 2)** | | | **Mean larval and pupal mortality D3 – D22 [%]** | | **Adult emergence [%]** |
| **absolute** | **corrected 1)** | **absolute** | | **corrected 1)** | **absolute** | **corrected 1)** |
| **Control** | **--** | **--** | 8.3 | -- | 0.0 | 12.1 | | -- | 19.4 | -- | 80.6 |
| **BAS 650 00 F** | 37.1 | 241 | 2.8 | 0.0 | 0.0 | 31.4 | | 22.0 | 33.3 | 17.2 | 66.7 |
| 92.6 | 602 | 0.0 | 0.0 | 0.0 | 61.1 | | 55.7 | 61.1 | 51.7 | 38.9 \* |
| 231.6 | 1504 | 8.3 | 0.0 | 0.0 | 72.7 | | 69.0 | 75.0 | 69.0 | 25.0 \* |
| 579.0 | 3760 | 25.0 | 18.2 | 0.0 | 77.8 | | 74.7 | 83.3 | 79.3 | 16.7 \* |
| 1447.6 | 9400 | 13.9 | 6.1 | 0.0 | 90.3 | | 89.0 | 91.7 | 89.7 | 8.3 \* |
| **Endpoints** | | | | | | | | | | | |
| **Larval mortality (D8)** | | | | | | | | | | | |
| **Test item dose** | | **BAS 650 00 F** | | | | | **a.s.** | | | | |
| **LD50 [µg/larva] 3)** | | > 1447.6 | | | | | > 277.6 | | | | |
| **NOED [µg/larva] 4)** | | ≥ 1447.6 | | | | | ≥ 277.6 | | | | |
| **Test item concentration** | | **BAS 650 00 F** | | | | | **a.s.** | | | | |
| **LC50 [mg/kg food] 3)** | | > 9400 | | | | | > 1802 | | | | |
| **NOEC [mg/kg food] 4)** | | ≥ 9400 | | | | | ≥ 1802 | | | | |
| **Adult emergence (D22)** | | | | | | | | | | | |
| **Test item dose** | | **BAS 650 00 F** | | | | | **a.s.** | | | | |
| **ED50 [µg/larva] (95% CL) 5)** | | 120.3 (82.4 – 175.4) | | | | | 23.1 (15.8 – 33.6) | | | | |
| **NOED [µg/larva] 6)** | | 37.1 | | | | | 7.1 | | | | |
| **Test item concentration** | | **BAS 650 00 F** | | | | | **a.s.** | | | | |
| **EC50 [mg/kg food] (95% CL) 5)** | | 781 (536 – 1140) | | | | | 149 (102 – 218) | | | | |
| **NOEC [mg/kg food] 6)** | | 241 | | | | | 46 | | | | |

OO: Other observations (large quantities of remaining food and/or body size)

95% CL = upper / lower 95% confidence limits.

1) According to Schneider-Orelli 1947.

2) Mean mortality of pupae was calculated by the product of the number of dead pupae on D22 (death occurred between D8 and D22) and 100, divided by the number of larvae alive on D8 as being the baseline value.

3) Estimated.

4) Chi² 2x2 Table Test with Bonferroni Correction; α = 0.05, one sided greater.

5) Median effect dose/concentration of exposure was calculated using a Logit analysis (using linear max. likelihood regression).

6) Step-down Cochran-Armitage Test; α = 0.05; one sided greater.

\* Statistically significant difference in pairwise comparison between treatment and untreated control.

**C. VALIDITY CRITERIA**

The study fulfilled the validity criteria outlined in the study, as detailed below:

* Larval mortality should be ≤15% across all control replicates between day 3 and day 8.3% larval mortality was observed across the control replicates; therefore, this criterion was met.
* Adult emergence rate should be ≥70% across all control replicates between day 3 and day 22. 80.6% of bees emerged across all control replicates up to day 22, therefore this criterion was met.
* Larval mortality in the reference item treatment group should be ≥50% across all replicates. 72.2% mortality was observed in the reference item treatment group between day 3 and day 8, therefore this criterion was met.

1. **CONCLUSION**

In a repeated exposure larval toxicity study with BAS 650 00 F, the LD50 (larval mortality on D8) was determined to be > 1447.6 µg BAS 650 00 F/larva (> 277.6 µg a.s./larva), which is equivalent to an LC50 of > 9400 mg BAS 650 00 F/kg food (> 1802 mg a.s./kg food). The respective NOED was ≥ 1447.6 µg BAS 650 00 F/larva (≥ 277.6 µg a.s./larva) and the corresponding NOEC was ≥ 9400 mg BAS 650 00 F/kg food (≥ 1802 mg a.s./kg food).

In a repeated exposure larval toxicity study with BAS 650 00 F, the ED50 (successful adult emergence up to D22) was determined to be 120.3 µg BAS 650 00 F/larva (23.1 µg a.s./larva), which is equivalent to an EC50 of 781 mg BAS 650 00 F/kg food (149 mg a.s./kg food). The respective NOED was 37.1 µg BAS 650 00 F/larva (7.1 µg a.s./larva) and the corresponding NOEC was 241 mg BAS 650 00 F/kg food (46 mg a.s./kg food).

* + - * 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 239 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.1.3/02 |
| Report | Repeated exposure of honey bee (*Apis mellifera* L.) larvae to BAS 743 02 F under laboratory conditions  Schmidt, K., 2022  XXXX Study ID: 933752\_3  XXXX Doc ID: 2022/2033710 |
| Guideline(s): | OECD 239 (2021) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

In a feeding toxicity test with repeated exposure, honey bee larvae (*Apis mellifera* L.) were exposed to BAS 743 02 F diluted in the larval food. The toxicity of the test item was determined at total doses of 352.2, 117.3, 39.1, 13.0 and 4.4 µg product/larva (equivalent to concentrations of 2226.1, 741.3, 247.1, 82.4 and 27.6 mg product/kg food, respectively). Additionally, honey bee larvae were treated with Dimethoate tech. as reference item at a dose of 7.4 µg a.s./larva (concentration: 47 mg a.s./kg food). Untreated diet served as control. Three replicates were used per treatment, each containing 12 larvae, established from three different colonies. Assessments of larval mortality were done on days 4, 5, 6, 7 and 8. Additionally, other observations such as small body size or large quantities of remaining food on day 8 were noted. Pupal mortality was assessed at day 15 and emergence of adults was evaluated at day 22.

The 8-day LD50 was estimated to be > 352.2 µg product/larva, the highest dose tested, equivalent to LC50 > 2226.1 mg product/kg food. The respective NOED was estimated to be ≥ 352.2 µg product/larva and the corresponding NOEC was estimated to be ≥ 2226.1 mg product/kg food.

The 22-day ED50 (successful adult emergence) was calculated to be > 352.2 µg product/larva, the highest dose tested, equivalent to EC50 > 2226.1 mg product/kg food. The 22-day ED20 and ED10 values were determined to be 93.3 and 44.5 µg product/larva, respectively. The respective NOED was determined to be 39.1 µg product/larva and the corresponding NOEC was determined to be 247.1 mg product/kg food.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Description:** | SC (suspension concentrate) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Density:** | 1.080 g/cm³ |
| **Storage conditions:** | At room temperature (typically +5°C to +35°C) |
| **Stability (expiry date):** | 31.01.2024 |
| 1. **Control:** | Untreated larval diet |
| 1. **Reference item:** | Dimethoate tech. (analyzed purity: 99.32 % w/w) |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. **Test organism:** | *Apis mellifera* L. subspeciesBuckfast |
| **Age:** | One day old, first instar larvae |
| **Source:** | The colonies were reared in the test facility. All larvae used in the test derived from healthy (free of clinical symptoms of any disease) and queen-right bee colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month. |
| **Methods for producing the larvae:** | Three colonies were used in the test. Each of the three colonies used in the test was treated in parallel the same way: On day -3 the respective queen of the colony was caged on an empty brood comb, which was fitted in an excluder cage and thereafter placed in the hive. The queen laid her eggs solely on this comb. The caging time was approximately 25 hours. In the afternoon of day -2 the queen was released from the excluder. The comb was checked for the presence of freshly laid eggs, was confined in the excluder again in order to avoid any additional egg laying and was placed near to frames containing open brood in the hive. The eggs were incubated within the hive between day -2 and day 1. |
| **Diet:** | A sufficient amount of food was present in the bee hives. The aqueous yeast/sugar solutions as one component of the artificial diets were prepared and stored in a fridge until use on day 2, 4, 5 and 6. The sugar solution was mixed with royal jelly every day before each feeding occasion. Each larva was fed separately using a sterile pipette. The food drop was placed next to the larvae to avoid drowning. Before feeding, the final diets were warmed to about 34.5°C. During the process the culture plate in operation was placed on a warming plate to prevent the larvae from cooling down. Three different diets depending on developmental stage:  Diet A (day 1): 50% w/w fresh royal jelly + 50% w/w aqueous solution containing 2% w/v yeast extract, 12% w/v glucose and 12% w/v fructose  Diet B (day 3): 50% w/w fresh royal jelly + 50% w/w aqueous solution containing 3% w/v yeast extract, 15% w/v glucose and 15% w/v fructose  Diet C (days 4 – 6): 50% w/w fresh royal jelly + 50% w/w aqueous solution containing 4% w/v yeast extract, 18% % w/v glucose and 18% w/v fructose |
| 1. **Test units:** | Crystal polystyrene grafting cells (CNE Nicotplast, internal diameter 9 mm) placed in 48 well culture plates. |
| 1. **Environmental conditions** |  |
| **Temperature:** | 34.0 °C – 34.9 °C |
| **Relative humidity:** | D1 – D8: 93.4 – 100.0%  D8 – D15: 81.3 – 83.4%  D15 – D22: 52.1 – 65.4% |
| **Photoperiod:** | Darkness, except during observation |

1. **Test organism and treatment:**

The study was conducted at BioChem agrar (Labor für biologische und chemische Analytik GmbH Kupferstr. 6, 04827 Machern OT Gerichshain, Germany) between 19.09.2022 and 10.10.2022. First-instar (one day old) honeybee larvae of *Apis mellifera* L. were transferred from brood combs to crystal polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. On 4 successive days (day 3 to day 6) the larvae were chronically exposed to BAS 743 02 F diluted in the larvae’s food (aqueous sugar solution mixed with royal jelly). After the applications, no additional feedings of the larvae took place. The test duration was 22 days.

In total, seven treatment groups, each comprising 12 larvae per replicate, were set up: five doses of the test item BAS 743 02 F diluted in the larval food, one control (untreated diet) and one dose (7.4 µg a.s./larva) of the reference item Dimethoate tech. with three replicates per treatment group, respectively. The nominal doses of BAS 743 02 F used were: 352.2, 117.3, 39.1, 13.0 and 4.4µg product/larva (equivalent to 140.9, 46.9, 15.6, 5.2 and 1.7µg propamocarb/larva and 44.7, 14.9. 5.0, 1.7 and 0.6 µg ametoctradin/larva, respectively). The respective concentrations of test item in the diet were 2226.1, 741.3, 247.1, 82.4 and 27.6 mg product/kg food (equivalent 890.4, 296.5, 98.8, 32.9 and 11.0 mg propamocarb/kg food and 282.7, 94.1, 31.4, 10.5 and 3.5 mg ametoctradin/kg food).

1. **Dose preparation:**

A base stock solution was prepared by dissolving 0.128 g test item in the corresponding aqueous sugar solution (for diets on day 3/ days 4-6, respectively). Serial dilutions were conducted to prepare the lower stock solutions. The final diets were produced by mixing each stock solution with royal jelly at a ratio of 1:1 (based on w/w) in order to produce final diets. To ensure a homogenous distribution of the test item within the larval food, the final diets were placed on a multitube vortex shaker at 2500 rpm for 5 minutes at room temperature.

The reference item base stock solution was prepared by dissolving 0.048 g dimethoate tech. in in the corresponding aqueous sugar solution to a total volume of 100 mL. The reference stock solution was prepared by mixing the base stock solution with royal jelly at a weight ratio of 1:1 in order to produce final diet.

1. **Measurements and observations:**

Biological observations:

The number of dead larvae (an immobile larva or one which does not react to contact is noted as dead) was recorded daily from days 4 to 8 (larvae), on day 15 (pupae) and on day 22. “Larval mortality” includes all individuals which had died between days 3 and day 8, while dead individuals between day 8 and day 15 are summarised as “pupal mortality”. The sum of all dead individuals between day 3 and day 22 is described as “total mortality”.

The numbers of bees which emerged successfully were counted at test end, i.e. on day 22. In order to correct the effects observed in the treatment group by background mortality (observed as control mortality) any calculations were performed using “mortality” instead of “adult emergence”.

Large amounts of unconsumed food and/or discolourations and/or abnormal behavior and/or substantially undersized larvae on day 8 were notified in order to support in the interpretation of mortality data.

Physicochemical measurements:

Air temperature and relative humidity were recorded continuously during the study.

Analytical verification:

For verification of the exposure concentrations, all final diets were sampled in triplicate as specimens for analysis and retention directly before feeding on D3, D4, D5, and D6. Until analysis the specimens were stored at ≤ -18°C.

1. **Statistical analysis:**

Mortality of control and reference item on day 8 and day 22 were compared using the Fisher’s Exact Binominal Test (alpha = 0.05, one-sided greater). For statistical evaluation of the mortality results after test item treatment and for determination of NOEC/NOED the Fisher Test After Bonferroni-Holm (day 8) and the Step-down Cochran-Armitage Test (day 22) were used. The accepted significance level was p ≤ 0.05 (one-sided greater).

LD/LC50 values (day 8) were estimated based on the raw data (due to effects <50%, the values were estimated to be higher than the highest dose/concentration). ED/EC50/20/10 calculations on day 22 were performed with the Probit analysis using linear max. likelihood regression. The statistical calculations were performed with the computer program ToxRatPro 3.3.0 (Ratte, 2018).

1. **Description of the analytical procedures**

Methodology was validated to quantify the amount of BAS 743 02 F via its active ingredients Propamocarb and BAS 650 F present in recovery samples pre-pared in test item feeding solutions. The determination was conducted using reversed phase high performance liquid chromatography (RP-HPLC) with tandem mass spectrometric (MS/MS) detection. All samples were extracted prior to sample analysis with 50/50 (v/v) (acetonitrile + 0.5% (v/v) formic acid))/water. The resulting extracts were further diluted into the range of the calibration curve before injecting into the HPLC-system. The method was fully validated according to the requirements of SANTE/2020/12830, Rev.1 guideline. Five replicate dilutions were analyzed for each selected application solution. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 70 to 110%. The mean recoveries of BAS 743 02 F in final diets were in the range of 100% to 106% Propamocarb and 105% to 107% BAS 650 F. The analyzed untreated control samples showed no residues at or above the LOD. The limit of quantitation (LOQ) was 1.48 mg/kg for Propamocarb and 0.469 mg/kg for BAS 650 F. The limit of detection was 0.311 mg/kg for Propamocarb and 0.0988 mg/kg for BAS 650 F.

1. **Results and Discussion**
2. **biological effects**

Cumulative mortality

Larval mortality (day 8) in the control group was 0.0% while pupal mortality (between day 8 and 15) was 2.8%. The control group showed a total mortality of 16.7% on day 22. In the test item groups, larval mortalities at day 8 ranged between 0.0 and 13.9%. Pupal mortalities ranged between 6.1 and 23.3% in the test item treatment groups. Total mortalities on day 22 ranged between 11.1 and 55.6%. Mortality in the reference group was above 50% across all replicates on day 8, being 90.6%.

Based on the above-mentioned results, the LD50 was estimated to be > 352.2 µg product/larva, which is equivalent to an LC50 > 2226.1 mg product/kg food. The respective NOED was ≥ 352.2 µg product/larva and the corresponding NOEC was ≥ 2226.1 mg product/kg food.

Adult emergence success:

In the final assessment on day 22, an adult emergence rate of 83.3% was determined for the honey bees in the control group. In the test item treated groups, the adult honey bees emerged at rates of 44.4, 61.1, 77.8, 80.6 and 88.9% following an application of 352.2, 117.3, 39.1, 13.0 and 4.4 µg product/larva during the larval stages. On day 22, larvae treated with 352.2 and 117.3 µg product/larva, showed adult emergence, which was statistically significantly decreased if compared to the control.

Based on the observed emergence rates the ED50 was determined to be > 352.2 µg product/larva, which is equivalent to an EC50 of > 2226.1 mg product/kg food. The respective NOED was 39.1 µg product/larva and the corresponding NOEC was 247.1 mg product/kg food.

Other observations

On day 8, 2.8% of the remaining larvae in the control treatment and 14.4%, 3.3%, 9.1% and 2.8% of the remaining larvae treated with 352.2, 39.1, 13.0 and 4.4 µg product/larva in the test item treatment showed remaining food.

The biological results are summarised in following table.

Table A 57: Cumulated mortality and other observations of larvae in the chronic toxicity test

| **Treatment group** | **Dose**  **[µg prod./ larva]** | **Concentration**  **[mg prod./kg food]** | **On D8** | | | **On D15** | | **On D22** | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Larval mortality**  **D3 to D8**  **[%]** | | **Mean OO**  **[%]** | **Pupal mortality**  **D8-D15**  **[%]** | | **Pupal mortality**  **D8-D22**  **[%]** | | **Total mortality**  **D3-D22**  **[%]** | | **Adult emergence**  **[%]** |
| abs. | corr. |  | abs. | corr. | abs. | corr. | abs. | corr. | abs. |
| **Control** | - | - | 0.0 | - | 2.8 | 2.8 | 0.0 | 16.7 | 0.0 | 16.7 | 0.0 | 83.3 |
| **Test item** | 352.2 | 2226.1 | 13.9 | - | 14.4 | 23.3 | 21.1 | 50.6 | 40.7 | 55.6 | 46.7 | 44.4\* |
| 117.3 | 741.3 | 5.6 | - | 0.0 | 8.8 | 6.2 | 35.6 | 22.7 | 38.9 | 26.7 | 61.1\* |
| 39.1 | 247.1 | 5.6 | - | 3.3 | 8.9 | 6.3 | 17.8 | 1.3 | 22.2 | 6.7 | 77.8 |
| 13.0 | 82.4 | 5.6 | - | 9.1 | 6.1 | 3.4 | 14.9 | 0.0 | 19.4 | 3.3 | 80.6 |
| 4.4 | 27.6 | 0.0 | - | 2.8 | 8.3 | 5.7 | 11.1 | 0.0 | 11.1 | 0.0 | 88.9 |
| **Reference** | 7.4 | 47 | 80.6\* | - | 16.7 | 25.0 | 22.9 | 41.7 | 30.0 | 94.4 | 93.3 | 5.6\* |

corr.: corrected mortality (according to schneider-Orelli 1947); abs.: absolute; OO: Other observations (e.g. remaining food, smaller body size, discolourations); Calculations were performed with non-rounded values;

\* Statistically significant difference compared to the control (Fisher’s Exact Binomial Test and Step-down Cochran-Armitage Test Procedure)

Toxicity endpoints for BAS 743 02 F are summarised in following table.

Table A 58: Calculated endpoints of the repeated exposure larvae toxicity test

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Endpoint: larval mortality** | **D8 [product]** |
| Test item doses | LD50 [µg /larva] 3) | > 352.2 |
| NOED [µg /larva] 1) | ≥ 352.2 |
| Test item  concentrations | LC50 [mg /kg food] 3) | > 2226.1 |
| NOEC [mg /kg food] 1) | ≥ 2226.1 |
| **Treatment** | **Endpoint: Successful adult  emergence** | **Up to D22 [product]** |
| Test item doses | ED50 [µg /larva] 4) (95% CL) | > 352.2 |
| ED20 [µg /larva] 4 (95% CL) | 93.3 (60.4 – 144.2) |
| ED10 [µg /larva] 4) (95% CL) | 44.5 (25.6 – 77.6) |
| NOED [µg /larva] 2) | 39.1 |
| Test item  concentrations | EC50 [mg /kg food] 4) (95% CL) | > 2226.1 |
| EC20 [mg /kg food] 4) (95% CL) | 589.8 (381.8 – 911.2) |
| EC10 [mg /kg food] 4) (95% CL) | 281.5 (161.5 – 490.6) |
| NOEC [mg /kg food] 2) | 247.1 |

1 Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm; α=0.05; one-sided greater

2 Step-down Cochran-Armitage Test Procedure; α=0.05; one-sided greater

3 due to effects <50%, the values were estimated to be higher than the highest dose/concentration

4 Probit analysis using linear maximum likelihood regression

CL: confidence limits

1. **ANALYTICAL RESULTS**

Table A 59: Measured concentrations of Propamocarb and Ametoctradin in test feeding solutions

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Propamocarb** | | | **Ametoctradin** | | |
| **Nominal Concentration**  **[mg/kg]** | **Mean Residue Concentration [mg/kg]** | **Mean Recovery**  **[%]** | **Nominal Concentration**  **[mg/kg]** | **Mean Residue Concentration [mg/kg]** | **Mean Recovery**  **[%]** |
| AC | 0.000 | <LOD | - | 0.000 | <LOD | - |
| ET | 11.0 | 11.7 | 106 | 3.51 | 3.75 | 107 |
| DT | 32.9 | 34.1 | 103 | 10.5 | 11.0 | 105 |
| CT | 98.8 | 102 | 103 | 31.4 | 32.9 | 105 |
| BT | 297 | 296 | 100 | 94.1 | 98.5 | 105 |
| AT | 890 | 908 | 102 | 283 | 301 | 106 |

LOQ: 1.48 mg/kg Propamocarb, LOQ: 0.469 mg/kg BAS 650 F

LOD: 0.311 mg/kg Propamocarb, LOD: 0.0988 mg/kg BAS 650 F

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 60: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 239 (2016)** | **Obtained in this study** |
| In the control plate(s), cumulative larval mortality from D3 to D8 should be ≤15% across all replicates. | 0.0% |
| In the control plate(s), the adult emergence rate on D22 should be ≥70% across all replicates. | 83.3% |
| Positive control: if dimethoate is used, larval mortality should be ≥50% on D8 across all replicates. | 80.6% |

1. **DEFICIENCIES**

There were no deviations with impact on quality and integrity of the study.

1. **Conclusion**

In a repeated exposure larval toxicity study with BAS 743 02 F, the 8- day LD50 was estimated to be > 352.2 µg product/larva, corresponding to an LC50 of > 2226.1 mg product/kg food. The respective NOED was ≥ 352.2 µg product/larva and the corresponding NOEC was ≥ 2226.1 mg product/kg food.

The 22-day ED50 (successful adult emergence) was determined to be > 352.2 µg product/larva, corresponding to an EC50 of > 2226.1 mg product/kg food. The 22-day ED20 and ED10 values were determined to be 93.3 and 44.5 µg product/larva, respectively. The respective NOED was 39.1 µg product/larva and the corresponding NOEC was 247.1 mg product/kg food.

* + - 1. KCP 10.3.1.4 Sub-lethal effects

As BAS 743 03 F does not pose an unacceptable risk to honeybees, further tests are not necessary.

* + - 1. KCP 10.3.1.5 Cage and tunnel tests

No data submitted.

* + - 1. KCP 10.3.1.6 Field tests with honeybees

No data submitted.

* + 1. KCP 10.3.2 Effects on non-target arthropods other than bees
       1. KCP 10.3.2.1 Standard laboratory testing for non-target arthropods
          1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.2.1/01 |
| Report | Effects of BAS 743 02 F on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test  Röhlig, U., 2022a  XXXX Study ID: 933752\_16  XXXX Doc ID: 2022/2033725 |
| Guideline(s): | IOBC (Blümel et al. 2000) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

A study to determine the effects of BAS 743 02 F (active substances Propamocarb and BAS 650 F) on protonymphs of the predatory mite *Typhlodromus pyri* was performed over 14 days in the laboratory. Protnymphs were exposed to freshly dried residues of the test item on glass plates. The test comprised seven treatment groups; five nominal test item rates of 0.1875, 0.375, 0.75, 1.5 and 3 L product/ha, a water control and toxic reference item group, with fvie replicates (consisting of 20 protonymphs) per treatment group. Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 9, 11 and 14 days. In addition, for all test item rates, and the control, the reproduction, i.e. number of eggs per female, was determined in three assessments at 9, 11 and 14 days after application.

The LR50 for *Typhlodromus pyri* was estimated to be > 3 L product/ha and the NOER for mortality was determined to be 0.375 L product/ha. The ER50 was estimated to be > 3 L product/ha and the NOER for reproduction was determined to be 3 L product/ha.

**I. MATERIALS AND METHODS**

1. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Description:** | Liquid, soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Density:** | 1.080 g/cm3 |
| 1. **Control:** | Purified water |
| 1. **Reference item:** | DANADIM PROGRESS, a.s. dimethoate, nominal 400 g/L, analysed 401.7 g/L |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) |
| **Age/life stage:** | Protonymphs (< 24 hours old) |
| **Source:** | Purchased from “Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany |
| **Diet:** | Pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1 |
| 1. **Test units:** | Two glass plates (cover glasses: 50 mm × 22 mm stuck together along their longitudinal sides) with a barrier of sticky glue (Temmen-Insektenleim, Temmen GmbH, Germany) on moistened filter paper on a sponge placed in a plastic tray (inside dimensions: about 165 mm × 120 mm x 60 mm) filled with tap water up to a height of approx. 15 mm |
| 1. **Environmental conditions** |  |
| **Temperature:** | 24 – 27°C |
| **Relative humidity:** | 70 – 86% |
| **Photoperiod:** | 16 h light : 8 h dark; 2070 lux |

1. **Test organism and treatment:**

The study comprised seven treatment groups; five nominal application rates of the test item, a water control and a toxic reference. There were five replicate arenas per treatment, each containing 20 protonymphs. The test item rates and their respective active substance contents are summarised in Table A 61.

Table A 61: Test item rates and active substance content

| **BAS 743 02 F (L product/ha)** | **Propamocarb**  **(g a.s./ha)1** | **Ametoctradin**  **(g a.s./ha)1** |
| --- | --- | --- |
| 0.1875 | 81 | 25.714 |
| 0.375 | 162 | 51.428 |
| 0.75 | 324 | 102.855 |
| 1.5 | 648 | 205.71 |
| 3 | 1296 | 411.42 |

1Based on nominal content of active substances

The treatments were applied to glass plates from directly above using a calibrated laboratory track-sprayer and the residues were left to dry. The spray pressure selected was 3.4 bar and the moving spray boom was fitted with a single 90° flat-fan nozzle (Lechler ES 90-015). All treatments were applied at a volume rate of 200 L spray solution/ha.

Approximately 1 hour after treatment, the test units were assembled along their longitudinal side parallel to one another and touching. The treated surface was placed upwards on moistened filter paper on a sponge placed in a plastic tray. A band of sticky insect glue was applied around the edges of the arena to form a barrier to prevent the mites from escaping. Then the protonymphs were put in the test arena by means of a moistened brush. The test apparatus was placed in a controlled environment room.

1. **Dose preparation:**

The volume application rate for the test was 200 L spray solution/ha. All dilutions were prepared shortly before their application. A stock solution, also used as the highest test item solution, was prepared by dissolving 1.620 g BAS 743 02 F in 100 mL deionised water. Serial dilutions were conducted to prepare the lower test item spray solutions.

1. **Measurements and observations:**

Biological observations:

Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 9, 11 and 14 days. Mites were classified as surviving, dead and escaped (trapped or not found). In addition, for all test item treatments, and the control, the reproduction, i.e. number of eggs laid per female, was determined in three assessments at 9, 11 and 14 days after application.

Physicochemical measurements:

Light levels were recorded at the start of the test and temperature and humidity measurements were taken continuously throughout the test.

1. **Statistical analysis:**

For statistical analysis of the results, the computer program ToxRat Professional 3.3.0 (RATTE, 2018) was used. Mortality was analysed for statistical significance using the Step-down Cochran-Armitage Test for the test item and the Chi2-2x2 Table test for reference item as distribution-free tests (α = 0.05, one-sided greater) which do not require testing for normality or homoscedasticity prior to analysis. Reproduction was analysed for statistical significance using Williams-t-test, α = 0.05, one-sided smaller, following Shapiro-Wilk’s test for normal distribution, Levene’s test procedure for variance homogeneity.

Since there were only low effects (< 50 %) for mortality and reproduction assessments in the test item treatment groups, the calculation of the LR50 and ER50 was not possible.

**II. Results and Discussion**

1. **biological effects**

After 7 days, a mean mortality of 1.0 % was observed in the control. In the test item treatments, mean (corrected) mortalities ranged between 1.0 % and 35.4 %. No statistically significant effects on mortality were determined at treatment rates up to and including 0.375 L product/ha compared to the control. The LR50 was estimated to be > 3 L product/ha. The NOER for mortality was determined to be 0.375 L product/ha. The reference item caused 76.0 % mortality in exposed mites, resulting in a corrected mortality of 75.8 %.

The mean reproduction rate in the control was 6.36 eggs/female. The mean reproduction rates in the test item treated groups were between 6.13 and 6.59 eggs/female. Thus, an effect on reproduction between - 3.6 % and 3.6 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at all test item rates compared to the control. The ER50 was estimated to be > 3 L product/ha and the NOER for reproduction was determined to be 3 L product/ha.

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Results are summarised in Table A 62.

Table A 62: Effects on predatory mite *Typhlodromus pyri* exposed to residues of BAS 743 02 F in a worst-case laboratory test

| **Treatment** | **Rate1** | **Mortality (%)** | **Corrected mortality2 (%)** | **Reproduction3**  **(mean number of eggs/female)** | **Inhibition of reproduction5 (%)** |
| --- | --- | --- | --- | --- | --- |
| Control | - | 1.0 | - | 6.36 | - |
| BAS 743 02 F  [L product/ha] | 0.1875 | 2.0 n.s. | 1.0 | 6.59 n.s. | -3.6 |
| 0.375 | 2.0 n.s. | 1.0 | 6.38 n.s. | -0.3 |
| 0.75 | 15.0\* | 14.1 | 6.51 n.s. | -2.4 |
| 1.5 | 24.0\*. | 23.2 | 6.47 n.s. | -1.7 |
| 3 | 36.0\* | 35.4 | 6.13 n.s. | 3.6 |
| Danadim Progress  [mL product/ha] | 15 | 76.0\* | 75.8 | n.d. | - |
| **Endpoint** [**L product/ha**] | | | | | |
| LR50 | >3 | | | | |
| NOER | 0.375 | | | | |
| ER50 | >3 | | | | |
| NOER | 3 | | | | |

1 Application rate in 200 L water/ha

2 Corrected mortality according to Abbott (1925)

3Results for reproduction compared by Williams-t-test (α = 0.05, one-sided smaller)

4 Reproduction performance, relative to control. A positive value indicates a lower reproduction performance and a negative value indicates a higher reproduction performance relative to the control.

n.s. Not statistically significantly different compared to the control

\* Statistically significantly different compared to the control. The results for mortality in individual test item treatments were compared to those in the control using Step-down Cochran-Armitage Test (α = 0.05, one-sided greater) (test item) and Chi2-2x2 Table test (α = 0.05, one-sided greater) (reference item).

In the toxic reference treatment, 76% mortality was observed in exposed mites (75.8% corrected mortality), which demonstrated the sensitivity of the test system.

1. **VALIDITY CRITERIA**

All validity criteria were met (Table A 63).

Table A 63: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| In the control(s), mortality should be ≤20% (dead and escaped mites) on day 7 | 1.0% |
| Mortality in the toxic reference treatment should be ≥50% on day 7 | 75.8% |
| Mean reproduction in the control group should be ≥4 eggs per female | 6.36 |

1. **DEFICIENCIES**

None.

**III. Conclusion**

A study to determine the effects of BAS 743 02 F on protonymphs of the predatory mite *Typhlodromus pyri* was performed over 14 days in the laboratory. Protnymphs were exposed to freshly dried residues of the test item on glass plates. Effects on mortality and reproduction were subsequently assessed. The LR50 for *Typhlodromus pyri* was estimated to be > 3 L product/ha. The NOER for mortality was determined to be 0.375 L product/ha. The ER50 was estimated to be > 3 L product/ha and the NOER for reproduction was determined to be 3 L product/ha.

* + - * 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.2.1/02 |
| Report | Effects of BAS 743 02 F on the parasitic wasp *Aphidius rhopalosiphi* (DESTEPHANI-PEREZ) in a laboratory test  Röhlig, U., 2022b  XXXX Study ID: 933752\_17  XXXX Doc ID: 2022/2033728 |
| Guideline(s): | IOBC (Mead-Briggs et al. 2000) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

A study to determine the effects of BAS 743 02 F on the parasitic wasp *Aphidius rhopalosiphi* was performed over 14 days in the laboratory. Wasps were exposed to freshly dried residues of the test item on glass plates for 48 hours. The test comprised seven treatment groups; five nominal test item rates of 0.1875, 0.375, 0.75, 1.5 and 3 L product/ha, a water control and toxic reference item. There were four replicate arenas per treatment, each with 10 wasps (seven females and three males). Assessments of wasp mortality were made after 2, 24 and 48 hours. After 48 hours, surviving wasps (15 females per treatment group) were removed and their reproductive capacity was assessed by confining them individually for 24 hours in reproduction test units. Assessment of reproduction capacity, i.e. number of mummies per female, was made after 14 days.

The 48-hour LR50 and the 14-day ER50 were estimated to be > 3 L product/ha. The NOER for mortality and reproduction were determined to be 3 L product/ha.

**I. MATERIALS AND METHODS**

1. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Description:** | Liquid, soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Density:** | 1.080 g/cm3 |
| 1. **Control:** | Purified water |
| 1. **Reference item:** | DANADIM PROGRESS, a.s. dimethoate, nominal 400 g/L, measured 401.7 g/L |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Parasitic wasp *Aphidius rhopalosiphi* (Destephani-Perez)(Hymenoptera, Braconidae) |
| **Age/life stage:** | Adults <48 hours old at test start |
| **Source:** | Purchased from “Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany, (in the stage of mummies) |
| **Diet:** | Cotton wool soaked with a 1:3 v/v solution of honey and water using capillary force |
| 1. **Test units:** | Mortality assessment: test arena, made of 2 square glass plates (13 cm × 13 cm), held apart by an aluminium frame (13 cm × 13 cm × 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/min). Both glass plates were fitted with the treated surface inwards directed onto a square aluminium frame as floor and ceiling. Three sides of the frame contained six ventilation holes each (1 cm diameter). The inside surface of the frame was coated with black tight cotton material to seal the ventilation holes. The fourth side of the frame contained an oval hole which was used for the introduction of the wasps and closed from the outside with black paper and adhesive tape.  Reproduction assessment: acrylic cylinders (about 11 cm Ø, 20 cm high), containing a pot grown with wheat seedlings and itself infested with > 100 adult and nymphal aphids of *Rhopalosiphum padi* and covered with gauze at the top of the cylinder. |
| 1. **Environmental conditions** |  |
| **Temperature:** | 20 – 22°C |
| **Relative humidity:** | 66 – 74% |
| **Photoperiod:** | 16 h light : 8 h dark; 1050 lux (exposure phase), 2670 lux (parasitisation phase), 6520 lux (reproduction phase) |

1. **Test organism and treatment:**

The study comprised seven treatment groups; five nominal application rates of the test item, a water control and a toxic reference. There were four replicate arenas per treatment, each containing 10 wasps with seven females and three males per replicate (i.e. a total of 40 per treatment). The test item rates and their respective active substance contents are summarised in Table A 64.

Table A 64: Test item rates and active substance content

| **BAS 743 02 F (L product/ha)** | **Propamocarb**  **(g a.s./ha)1** | **Ametoctradin**  **(g a.s./ha)1** |
| --- | --- | --- |
| 0.1875 | 81 | 25.714 |
| 0.375 | 162 | 51.428 |
| 0.75 | 324 | 102.855 |
| 1.5 | 648 | 205.71 |
| 3 | 1296 | 411.42 |

1Based on nominal content of active substances

The treatments were applied to glass plates from directly above using a calibrated laboratory track-sprayer and the residues were left to dry. The spray pressure selected was 3.4 bar and the moving spray boom was fitted with a single 90° flat-fan nozzle (Lechler ES 90-015). All treatments were applied at a volume rate of 200 L spray solution/ha.

Approximately 1 hour after treatment, the test units were assembled with the treated surfaces facing inwards (top and bottom). The wasps were transferred into each arena using an aspirator. The sex of the individual wasps was determined beforehand. The test apparatus was placed in a controlled environment room.

1. **Dose preparation:**

The volume application rate for the test was 200 L spray solution/ha. All dilutions were prepared shortly before their application. A stock solution, also used as the highest test item solution, was prepared by dissolving 1.620 g BAS 743 02 F in 100 mL deionised water. Serial dilutions were conducted to prepare the lower test item spray solutions.

1. **Measurements and observations:**

Biological observations:

The condition of the wasps was recorded after approximately 2, 24 and 48 hours. Wasps were classified as alive, affected, moribund or dead. After 48 hours, any moribund wasps were included with the dead insects for calculations of percentage mortality. In addition, for all test item treatments, and the control, the reproduction, i.e. number of parasitized aphids (mummies), was determined 14 days after application.

Physicochemical measurements:

Light levels were recorded at the start of the test and temperature and humidity measurements were taken continuously throughout the test.

1. **Statistical analysis:**

For statistical analysis of the results, the computer program ToxRat Professional 3.3.0 (RATTE, 2018) was used. Mortality was analysed for statistical significance using the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (test item) and Chi2 2x2 Table Test (reference item) as distribution-free tests (α = 0.05, one-sided greater) which do not require testing for normality or homoscedasticity prior to analysis. Reproductive capacity was analysed for statistical significance using Williams Multiple Sequential t-Test (α = 0.05, one-sided smaller) following Shapiro-Wilk’s test on normal distribution, Levene’s test on variance homogeneity.

Since there were only low effects (< 50 %) for mortality and reproduction in the test item treatment groups, the calculation of the LR50 and ER50 was not possible.

**II. Results and Discussion**

1. **biological effects**

After 48 hours, in the water-treated control, a mean mortality of 0 % was observed. In the test item treatments, the mean mortality ranged between 0 and 2.5 %. No statistically significantly increased mortalities were determined in all test item rates compared to the control. The LR50 was estimated to be > 3 L product/ha and the NOER for mortality was determined to be 3 L product/ha. The reference item caused 100 % mortality in exposed wasp.

The mean numbers of mummies produced per female in the respective test item treatment groups were between 15.8 and 17.8, compared to the control value of 17.1 mummies/female. No statistically significantly different reproduction rates were observed in all the test item rates compared to the control. The ER50 was estimated to be > 3 L product/ha and the NOER for reproduction was determined to be 3 L product/ha. No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Results are summarised in Table A 65.

Table A 65: Effects on the parasitic wasp *Aphidius rhopalosiphi* exposed to residues of BAS 743 02 F in a worst-case laboratory test

| **Treatment** | **Rate1** | **Mortality (%)** | **Corrected mortality2 (%)** | **Reproduction3**  **(mean number of mummies/female)** | **Inhibition of reproduction4 (%)** |
| --- | --- | --- | --- | --- | --- |
| Control | - | 0 | - | 17.1 | - |
| BAS 743 02 F  [L product/ha] | 0.1875 | 0 n.s. | 0 | 17.8 n.s. | -4.1 |
| 0.375 | 0 n.s. | 0 | 15.9 n.s. | 7.0 |
| 0.75 | 2.5 n.s. | 2.5 | 17.3 n.s. | -1.2 |
| 1.5 | 2.5 n.s. | 2.5 | 15.8 n.s. | 7.6 |
| 3 | 2.5 n.s. | 2.5 | 16.0 n.s. | 6.4 |
| Danadim Progress  [mL product/ha] | 0.3 | 100\* | 100 | n.d. | - |
| **Endpoint** [**L product/ha**] | | | | | |
| LR50 | >3 | | | | |
| NOER | 3 | | | | |
| ER50 | >3 | | | | |
| NOER | 3 | | | | |

1 Application rate in 200 L water/ha

2 Corrected mortality according to ABBOTT (1925).

3 Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results for the test item treatments and control were compared by Williams Multiple Sequentially t-Test (α = 0.05, one-sided smaller).

4 Reduction of the reproduction performance relative to the control. A positive value indicates a lower reproduction performance and a negative value indicates a higher reproduction performance relative to the control.

n.s. Not statistically significantly different compared to the control

\* Statistically significantly different compared to the control: Chi2 2x2 Table Test (α = 0.05, one-sided greater) for reference item

In the toxic reference treatment, 100% mortality was observed after 48 hours which demonstrated the sensitivity of the test system.

1. **VALIDITY CRITERIA**

All validity criteria were met (Table A 66).

Table A 66: Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| In the control(s), mortality should be ≤13% (i.e. 5 wasps from 40) after 48 hours | 0% |
| Mortality in the toxic reference treatment should be ≥50% after 48 hours | 92.5% |
| Mean reproduction in the control group should be ≥5 mummies per female\* | 17.1 with 1 control replicate with zero values |

\*There should be no more than 2 control replicates (with surviving wasps) with zero values

1. **DEFICIENCIES**

None.

**III. Conclusion**

A study to determine the effects of BAS 743 02 F on the parasitic wasp *Aphidius rhopalosiphi* was performed over 14 days in the laboratory. Wasps were exposed to freshly dried residues of the test item on glass plates for 48 hours. Effects on reproduction were subsequently assessed by the number of parasitised aphids (mummies) produced per surviving female. The 48-hour LR50 was estimated to be > 3 L product/ha and the NOER for mortality was determined to be 3 L product/ha. The ER50 was estimated to be > 3 L product/ha and the NOER for reproduction was determined to be 3 L product/ha.

* + - * 1. Study 3

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.3.2.1/03 |
| Report | Effects of BAS 743 03 F on the parasitic wasp *Aphidius rhopalosiphi* (DESTEPHANI-PEREZ) in a laboratory test  Röhlig, U., 2022c  XXXX Study ID: 933750\_4  XXXX Doc ID: 2022/2033732 |
| Guidelines: | IOBC (Mead-Briggs et al. 2000) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

A study to determine the effects of BAS 743 03 F on the parasitic wasp *Aphidius rhopalosiphi* was performed over 14 days in the laboratory. Wasps were exposed to freshly dried residues of the test item on glass plates for 48 hours. The test comprised seven treatment groups; five nominal test item rates of 0.2875, 0.575, 1.15, 2.3 and 4.6 L product/ha, a water control and toxic reference item. There were four replicate arenas per treatment, each with 10 wasps (seven females and three males). Assessments of wasp mortality were made after 2, 24 and 48 hours. After 48 hours, surviving wasps (15 females per treatment group) were removed and their reproductive capacity was assessed by confining them individually for 24 hours in reproduction test units. Assessment of reproduction capacity, i.e. number of mummies per female, was made after 14 days.

The 48-hour LR50 and the 14-day ER50 were estimated to be > 4.6 L product/ha. The NOER for mortality and reproduction were determined to be 4.6 L product/ha.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 03 F, |
| **Description:** | Liquid, soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002223 |
| **Active substance content:** | Propamocarb: nominal 378.0 g/L, analysed 376.7 g/L;  Ametoctradin: nominal 120.0 g/L, analysed 120.2 g/L |
| **Density:** | 1.071 g/cm3 at 20°C |
| 1. **Control:** | Deionised water |
| 1. **Reference item:** | DANADIM PROGRESS, a.s. dimethoate, nominal 400 g/L, measured 401.7 g/L |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Parasitic wasp *Aphidius rhopalosiphi* (Destephani-Perez)(Hymenoptera, Braconidae) |
| **Age/life stage:** | Adults <48 hours old at test start |
| **Source:** | Purchased from “Katz Biotech AG”, An der Birkenpfuhlheide 10, 15837 Baruth, Germany, (in the stage of mummies) |
| **Diet:** | Cotton wool soaked with a 1:3 v/v solution of honey and water using capillary force |
| 1. **Test units:** | Mortality assessment: test arena, made of 2 square glass plates (13 cm × 13 cm), held apart by an aluminium frame (13 cm × 13 cm × 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/min). Both glass plates were fitted with the treated surface inwards directed onto a square aluminium frame as floor and ceiling. Three sides of the frame contained six ventilation holes each (1 cm diameter). The inside surface of the frame was coated with black tight cotton material to seal the ventilation holes. The fourth side of the frame contained an oval hole which was used for the introduction of the wasps and closed from the outside with black paper and adhesive tape.  Reproduction assessment: acrylic cylinders (about 11 cm Ø, 20 cm high), containing a pot grown with wheat seedlings and itself infested with > 100 adult and nymphal aphids of *Rhopalosiphum padi* and covered with gauze at the top of the cylinder. |
| 1. **Environmental conditions** |  |
| **Temperature:** | 20 – 22°C |
| **Relative humidity:** | 66 – 75% |
| **Photoperiod:** | 16 h light : 8 h dark; 1020 lx (exposure phase), 2590 lx (parasitisation phase), 6410 lx (reproduction phase) |

1. **Test organism and treatment:**

The study comprised seven treatment groups; five nominal application rates of the test item, a water control and a toxic reference. There were four replicate arenas per treatment, each containing 10 wasps with seven females and three males per replicate (i.e. a total of 40 per treatment). The test item rates and their respective active substance contents are summarised in Table A 67.

Table A 67: Test item rates and active substance content

| **BAS 743 02 F (L product/ha)** | **Propamocarb**  **(g a.s./ha)1** | **Ametoctradin**  **(g a.s./ha)1** |
| --- | --- | --- |
| 0.2875 | 108.675 | 34.5 |
| 0.575 | 217.35 | 69 |
| 1.15 | 434.7 | 138 |
| 2.3 | 869.4 | 276 |
| 4.6 | 1738.8 | 552 |

1Based on nominal content of active substances

The treatments were applied to glass plates from directly above using a calibrated laboratory track-sprayer and the residues were left to dry. The spray pressure selected was 3.4 bar and the moving spray boom was fitted with a single 90° flat-fan nozzle (Lechler ES 90-015). All treatments were applied at a volume rate of 200 L spray solution/ha.

Approximately 1 hour after treatment, the test units were assembled with the treated surfaces facing inwards (top and bottom). The wasps were transferred into each arena using an aspirator. The sex of the individual wasps was determined beforehand. The test apparatus was placed in a controlled environment room.

1. **Dose preparation:**

The volume application rate for the test was 200 L spray solution/ha. All dilutions were prepared shortly before their application. A stock solution, also used as the highest test item solution, was prepared by dissolving 2.463 g BAS 743 03 F in 100 mL deionised water. Serial dilutions were conducted to prepare the lower test item spray solutions.

1. **Measurements and observations:**

Biological observations:

The condition of the wasps was recorded after approximately 2, 24 and 48 hours. Wasps were classified as alive, affected, moribund or dead. After 48 hours, any moribund wasps were included with the dead insects for calculations of percentage mortality. After 48 hours, surviving wasps (15 females per treatment group) were removed and their reproductive capacity, i.e. number of parasitized aphids (mummies), was determined 14 days after application.

Physicochemical measurements:

Light levels were recorded at the start of the test and temperature and humidity measurements were taken continuously throughout the test.

1. **Statistical analysis:**

For statistical analysis of the results, the computer program ToxRat Professional 3.3.0 (RATTE, 2018) was used. Mortality was analysed for statistical significance using the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (test item) and Chi2 2x2 Table Test (reference item) as distribution-free tests (α = 0.05, one-sided greater) which do not require testing for normality or homoscedasticity prior to analysis. Reproductive capacity was analysed for statistical significance using Williams Multiple Sequential t-Test (α = 0.05, one-sided smaller) following Shapiro-Wilk’s test on normal distribution, Levene’s test on variance homogeneity.

Since there were only low effects (< 50 %) for mortality and reproduction assessments in the test item treatment groups, the calculation of the LR50 and ER50 was not possible.

**II. Results and Discussion**

1. **biological effects**

After 48 hours, in the water-treated control, a mean mortality of 0 % was observed. In the test item treatments, the mean mortality ranged between 0 and 2.5 %. No statistically significantly increased mortalities were determined in all test item rates compared to the control. The LR50 was estimated to be > 4.6 L product/ha and the NOER for mortality was determined to be 4.6 L product/ha.

The mean numbers of mummies produced per female in the respective test item treatment groups were between 15.5 and 17.2, compared to the control value of 16.7 mummies/female. No statistically significantly different reproduction rates were observed in all the test item rates compared to the control. The ER50 was estimated to be > 4.6 L product/ha and the NOER for reproduction was determined to be 4.6 L product/ha. No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Results are summarised in Table A 68.

Table A 68: Effects on the parasitic wasp *Aphidius rhopalosiphi* exposed to residues of BAS 743 03 F in a worst-case laboratory test

| **Treatment** | **Rate1** | **Mortality (%)** | **Corrected mortality2 (%)** | **Reproduction3**  **(mean number of mummies/female)** | **Inhibition of reproduction4 (%)** |
| --- | --- | --- | --- | --- | --- |
| Control | - | 0 | - | 16.7 | - |
| BAS 743 02 F  [L product/ha] | 0.2875 | 0 n.s. | 0 | 16.9 n.s. | -1.2 |
| 0.575 | 0 n.s. | 0 | 16.2 n.s. | 3.0 |
| 1.15 | 2.5 n.s. | 2.5 | 17.2 n.s. | -3.0 |
| 2.3 | 2.5 n.s. | 2.5 | 15.5 n.s. | 7.2 |
| 4.6 | 2.5 n.s. | 2.5 | 17.1 n.s. | 2.4 |
| Danadim Progress  [mL product/ha] | 0.3 | 100\* | 100 | n.d. | - |
| **Endpoint** [**L product/ha**] | | | | | |
| LR50 | >4.6 | | | | |
| NOER | 4.6 | | | | |
| ER50 | >4.6 | | | | |
| NOER | 4.6 | | | | |

1 Application rate in 200 L water/ha

2 Corrected mortality according to ABBOTT (1925).

3 Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results for the test item treatments and control were compared by Williams Multiple Sequentially t-Test (α = 0.05, one-sided smaller).

4 Reduction of the reproduction performance relative to the control. A positive value indicates a lower reproduction performance and a negative value indicates a higher reproduction performance relative to the control.

n.s. Not statistically significantly different compared to the control

\* Statistically significantly different compared to the control: Chi2 2x2 Table Test (α = 0.05, one-sided greater) for reference item

In the toxic reference treatment, 100% mortality was observed after 48 hours which demonstrated the sensitivity of the test system.

1. **VALIDITY CRITERIA**

All validity criteria were met (Table A 69).

Table A 69 Validity criteria

| **Validity criteria according to the study plan** | **Obtained in this study** |
| --- | --- |
| In the control(s), mortality should be ≤13% (i.e. 5 wasps from 40) after 48 hours | 0% |
| Mortality in the toxic reference treatment should be ≥50% after 48 hours | 100% |
| Mean reproduction in the control group should be ≥5 mummies per female\* | 16.7 with 1 control replicate with zero values |

\*There should be no more than 2 control replicates (with surviving wasps) with zero values

1. **DEFICIENCIES**

None.

**III. Conclusion**

A study to determine the effects of BAS 743 03 F on the parasitic wasp *Aphidius rhopalosiphi* was performed over 14 days in the laboratory. Wasps were exposed to freshly dried residues of the test item on glass plates for 48 hours. Effects on reproduction were subsequently assessed by the number of parasitised aphids (mummies) produced per surviving female. The 48-hour LR50 was estimated to be > 4.6 L product/ha and the NOER for mortality was determined to be 4.6 L product/ha. The ER50 was estimated to be > 4.6 L product/ha and the NOER for reproduction was determined to be 4.6 L product/ha.

* + - 1. KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

No data submitted.

* + - 1. KCP 10.3.2.3 Semi-field studies with non-target arthropods

No data submitted.

* + - 1. KCP 10.3.2.4 Field studies with non-target arthropods

No data submitted.

* + - 1. KCP 10.3.2.5 Other routes of exposure for non-target arthropods

All relevant routes of exposure for non-target arthropods have been adequately covered in the submitted studies and the risk assessment performed.

* 1. KCP 10.4 Effects on non-target soil meso- and macrofauna
     1. KCP 10.4.1 Earthworms
        1. KCP 10.4.1.1 Earthworms - sub-lethal effects
           1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 222 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.4.1.1/01 |
| Report | Effects of BAS 743 02 F on the reproduction of the earthworm *Eisenia andrei* in artificial soil  Friedrich, S., 2022a  XXXX Study ID: 933752\_10  XXXX Doc ID: 2022/2033719 |
| Guideline(s): | OECD 222 (2016) |
| Deviations: | No |
| GLP: | Yes, (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany) |
| Acceptability: | Yes |

**Executive Summary**

A study to determine the effects of BAS 743 02 F on the mortality, biomass development and reproduction of the earthworm *Eisenia andrei* was performed over 56 days in artificial soil. The test comprised nine treatment groups; eight nominal test item concentrations between 16.3 and 1000 mg product/kg soil dry weight (dw) and an untreated control. Four replicates were used for the test item treatments and eight replicates were used for the control. Each replicate contained 10 earthworms. Assessment of adult worm mortality, behavioural effects and biomass development after 28 days was done. Reproduction after additional 28 days (56 days after application) was determined.

BAS 743 02 F did not show any statistically significant effects on mortality and body weight. Statistically significant effects on reproduction compared to the control were recorded at concentrations ≥ 309 mg product/kg soil dw. The NOEC for mortality and biomass was determined to be > 1000 mg product/kg soil dw, corresponding to a total amount of 527.0 mg a.s./kg soil dw (sum of active substances) respectively, 400.0 mg propamocarb/kg soil dw and 127.0 mg ametoctradin /kg soil dw. The NOEC for reproduction was determined to be 171 mg product/kg soil dw corresponding to a total amount of 90.4 mg a.s./kg soil dw (sum of active substances) respectively, 68.6 mg propamocarb/kg soil dw and 21.8 mg ametoctradin/kg soil dw. The EC10 and EC20 values for reproduction were considered not reliable and the EC50 value could not be determined but it can be concluded that it is > 1000 mg product/kg soil dw.

**I. MATERIALS AND METHODS**

1. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Description:** | Soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Density:** | 1.080 g/cm3 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Maypon Flow (Carbendazim, SC 500), investigated in a separate study |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Earthworm (*Eisenia andrei*) |
| **Age/life stage:** | Adults approx. 5 months old with clitellum |
| **Body weight:** | 300 – 499 mg |
| **Source:** | In-house culture, originally obtained from W. Neudorff GmbH KG |
| **Acclimatisation:** | At least 24 hours prior to test start in a separate batch of the artificial soil mixed with horse manure |
| **Diet:** | 1 day after application, 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was moistened with 5 mL deionised water. Earthworms were fed weekly during the first 4 weeks. The weekly amount of manure (5 g) was dependent on feeding activity, which was assessed by visual estimation of the food remaining on the surface. After adult worms were removed from the test vessels (4 weeks) the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion of the test. |
| 1. **Test units:** | Plastic vessels (inside dimensions approx. 16.5 cm × 12 cm × 6 cm) with a lid pervious to air and light. Approx. 810 g wet weight soil (600 g soil dw) per vessel. |
| 1. **Environmental conditions** |  |
| **Test soil:** | Artificial soil according to OECD 222: 10% sphagnum peat, 20% kaolin clay, 0.5% calcium carbonate and 69.5% industrial quartz sand |
| **Temperature:** | 19.8 – 21.9°C |
| **pH:** | Test start: 6.04 – 6.10  Test end: 5.68 – 5.79 |
| **WHCmax:** | 62.0 (g/100 g dry soil) |
| **Water content:** | Test start: 56.3 – 56.5% of WHCmax  Test end: 55.0 – 55.8% of WHCmax |
| **Photoperiod:** | 16 h light: 8 h dark (620 lux) |

1. **Test organism and treatment:**

The study comprised nine treatment groups (eight test item concentrations and a control). The test item concentrations and their respective active substance contents are summarised in Table A 70. Four replicates were used per test item treatment and eight replicates were used in the control. Each replicate contained 10 earthworms.

Table A 70: Active substance content of the test item at each test concentration

|  |  |  |
| --- | --- | --- |
| **BAS 743 02 F (mg/kg soil dw)** | **Propamocarb (mg a.s./kg soil dw)\*** | **Ametoctradin (BAS 650 F) (mg a.s./kg soil dw)\*** |
| 16.3 | 6.53 | 2.07 |
| 29.4 | 11.8 | 3.73 |
| 52.9 | 21.2 | 6.7 |
| 95.3 | 38.1 | 12.1 |
| 171 | 68.6 | 21.8 |
| 309 | 123.5 | 39.2 |
| 556 | 222.2 | 70.5 |
| 1000 | 400.0 | 127.0 |

\* The amounts of Propamocarb and BAS 650 F were calculated based on the nominal contents a.s. The density (1.080 g/cm³) was taken into account.

One day prior to test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content.

On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60% of its WHC. The control substrate contained the corresponding amount of deionised water only. Each test vessel was filled with the treated soil and groups of 10 worms were randomly assigned to each treatment group. After approximately 5 minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light but prevented worms from escaping. The test vessels were set up at random in a controlled-environment room.

1. **Dose preparation:**

An exact amount of the test item (10 g) was weighed and dispersed in deionised water to make up a final volume of 1000 mL, and the solution was mixed. This stock solution was diluted with deionised water to prepare further test solutions (serial dilution). Afterwards 60 mL of the test solutions were thoroughly mixed with the artificial soil (750 g wet weight) separately for each replicate by intensive stirring in a laboratory mixer.

1. **Measurements and observations:**

Biological observations:

Individual fresh weight of each adult worm was determined at the start of the test. Observations of behavioural and pathological symptoms (including feeding activity) were made weekly for the test duration. After 4 weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. At the final assessment after 8 weeks, the number of hatched juvenile earthworms in each test vessel was determined.

Physicochemical measurements:

Determination of water content and pH of the artificial soil was done at the start and end of the test.

1. **Statistical analysis:**

The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated.

The EC10, EC20 and EC50 values (number of juveniles) were calculated using the Logit analysis using the maximum likelihood method. Confidence limits (95 %) of the ECx values were computed by normal approximation. For identifying the NOEC values Williams-t-test and Dunnett-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

The statistical analysis was performed with the software ToxRat Professional 3.3.0. (2018).

**II. Results and Discussion**

1. **biological effects**

Mortality rates of 0 % were recorded in the test item treatment groups and in the control. No pathological symptoms and no further effects on behaviour of the worms were observed. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested.

Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations of 309, 556 and 1000 mg product/kg soil dw.

Table A 71: Summary of effects on mortality and reproduction of the earthworm *Eisenia andrei* following exposure to BAS 743 02 F

| **Treatment group** | **BAS 743 02 F (mg product/kg soil dw)** | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| Mortality (%) day 28 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Weight change (%) day 28 | 20.8 | 21.4 | 18.9 | 20.4 | 23.4 | 22.8 | 19.8. | 20.1 | 21.0 |
| Number of juveniles day 56 | 234.1 | 236.5 | 224.8 | 230.8 | 238.3 | 213.3 | 202.3\* | 182.8\* | 174.5\* |
| Reproduction in (%) of control | 100 | 101.0 | 96.0 | 98.6 | 101.8 | 91.1 | 86.4 | 78.1 | 74.5 |
|  | **Endpoint (mg product/kg soil dw)** | | | | | | | | |
| 28-day NOEC (mortality) | ≥ 1000 | | | | | | | | |
| 28-day NOEC (biomass) | ≥ 1000 | | | | | | | | |
| 56-day NOEC (reproduction) | 171 | | | | | | | | |
| 28-day LC50 (mortality)1 | > 1000 | | | | | | | | |
| 56-day EC10 (reproduction)2 | n.r. | | | | | | | | |
| 56-day EC20 (reproduction)2 | n.r. | | | | | | | | |
| 56-day EC50 (reproduction)2 | n.d. | | | | | | | | |

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

n.r.: values considered not reliable

\*: statistically significantly different from the control (Williams-t-test for reproduction, α = 0.05, one-sided smaller)

1 based on estimation of the data

2 based on Logit analysis.

In a separate study (BioChem project No. 22 48 TEC 0003, reported 28 Jan 2022), the reference item Maypon Flow (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of earthworms. The number of juveniles was reduced by 51.6 and 99.9 % at concentrations of 5 and 10 mg product/kg soil dw (mean number of juveniles = 130.5 and 0.3) after 8 weeks of test duration when compared to control (mean number of juveniles = 269.8). The results of the reference test demonstrated the sensitivity of the test system.

**B. VALIDITY CRITERIA**

All validity criteria were met.

Table A 72: Validity criteria

| **Validity criteria according to OECD 222 (2016)** | **Obtained in this study** |
| --- | --- |
| In the control(s), adult mortality after 4 weeks should be ≤10% | 0 % |
| In the control(s), number of juveniles per replicate should be ≥30 | 194 – 276 |
| In the control(s), coefficient of variation of reproduction should be ≤30% | 12.5% |

**C. DEFICIENCIES**

There were no deviations from the study plan.

**III. Conclusion**

In a 56-day earthworm *Eisenia andrei* reproduction study with BAS 743 02 F, no adverse effects on survival and biomass development could be determined at all concentrations tested up to and including 1000 mg product/kg soil dw. The NOEC for mortality and biomass was determined to be ≥1000 mg product/kg soil dw (corresponding to a total amount of 527.0 mg a.s./kg soil dw as sum of active substances). The NOEC for reproduction was determined to be 171 mg product/kg soil dw (corresponding to 90.4 mg a.s./kg soil dw as sum of active substances). The EC10 and EC20 values for reproduction were considered not reliable and the EC50 value could not be determined but it can be concluded that it is > 1000 mg product/kg soil dw. All validity criteria for the study were met.

* + - * 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 222 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.4.1.1/02 |
| Report | Effects of BAS 743 03 F on the reproduction of the earthworm *Eisenia andrei* in artificial soil  Friedrich, S., 2022b  XXXX Study ID: 933750\_3  XXXX Doc ID: 2022/2033731 |
| Guideline(s): | OECD 222 (2016) |
| Deviations: | No |
| GLP: | Yes, (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany) |
| Acceptability: | Yes |

**Executive Summary**

A study to determine the effects of BAS 743 03 F on the mortality, biomass development and reproduction of the earthworm *Eisenia andrei* was performed over 56 days in artificial soil. The test comprised nine treatment groups; eight nominal test item concentrations between 16.3 and 1000 mg product/kg soil dry weight (dw) and an untreated control. Four replicates were used for the test item treatments and eight replicates were used for the control. Each replicate contained 10 earthworms. Assessment of adult worm mortality, behavioural effects and biomass development after 28 days was done. Reproduction after additional 28 days (56 days after application) was determined.

BAS 743 02 F did not show any statistically significant effects on mortality and body weight. Statistically significant effects on reproduction compared to the control were recorded at concentrations ≥ 309 mg product/kg soil dw. The NOEC for mortality and biomass was determined to be ≥ 1000 mg product/kg soil dw. The NOEC for reproduction was determined to be 171 mg product/kg soil dw. The EC10 and EC20 values for reproduction were considered not reliable and the EC50 value could not be determined but it can be concluded that it is > 1000 mg product/kg soil dw.

**I. MATERIALS AND METHODS**

1. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 03 F |
| **Description:** | Soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002223 |
| **Active substance content:** | Propamocarb: nominal 378.0 g/L, analysed 376.7 g/L;  Ametoctradin: nominal 120.0 g/L, analysed 120.2 g/L |
| **Density:** | 1.080 g/cm3 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Maypon Flow (Carbendazim, SC 500), investigated in a separate study |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Earthworm (*Eisenia andrei*) |
| **Age/life stage:** | Adults approx. 5 months old with clitellum |
| **Body weight:** | 301 – 500 mg |
| **Source:** | In-house culture, originally obtained from W. Neudorff GmbH KG |
| **Acclimatisation:** | At least 24 hours prior to test start in a separate batch of the artificial soil mixed with horse manure |
| **Diet:** | 1 day after application, 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was moistened with 5 mL deionised water. Earthworms were fed weekly during the first 4 weeks. The weekly amount of manure (5 g) was dependent on feeding activity, which was assessed by visual estimation of the food remaining on the surface. After adult worms were removed from the test vessels (4 weeks) the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion of the test. |
| 1. **Test units:** | Plastic vessels (inside dimensions approx. 16.5 cm × 12 cm × 6 cm) with a lid pervious to air and light. Approx. 810 g wet weight soil (600 g soil dw) per vessel. |
| 1. **Environmental conditions** |  |
| **Test soil:** | Artificial soil according to OECD 222: 10% sphagnum peat, 20% kaolin clay, 0.5% calcium carbonate and 69.5% industrial quartz sand |
| **Temperature:** | 18.9 – 21.4°C |
| **pH:** | Test start: 5.95 – 6.04  Test end: 5.70 – 5.85 |
| **WHCmax:** | 62.0 (g/100 g dry soil) |
| **Water content:** | Test start: 56.3 – 56.5% of WHCmax  Test end: 55.3 – 56.1% of WHCmax |
| **Photoperiod:** | Test start: 5.95 – 6.04  Test end: 5.70 – 5.85 |

1. **Test organism and treatment:**

The study comprised nine treatment groups (eight test item concentrations and a control). The test item concentrations and their respective active substance contents are summarised in Table A 73. Four replicates were used per test item treatment and eight replicates were used in the control. Each replicate contained 10 earthworms.

Table A 73: Active substance content of the test item at each test concentration

|  |  |  |
| --- | --- | --- |
| **BAS 743 02 F (mg/kg soil dw)** | **Propamocarb (mg a.s./kg soil dw)\*** | **Ametoctradin (BAS 650 F) (mg a.s./kg soil dw)\*** |
| 16.3 | 5.76 | 1.83 |
| 29.4 | 10.4 | 3.29 |
| 52.9 | 18.7 | 5.93 |
| 95.3 | 33.6 | 10.7 |
| 171 | 60.5 | 19.2 |
| 309 | 108.9 | 34.6 |
| 556 | 196.1 | 62.2 |
| 1000 | 352.9 | 112.0 |

\* The amounts of Propamocarb and BAS 650 F were calculated based on the nominal contents a.s. The density (1.071 g/cm³) was taken into account.

One day prior to test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content.

On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60% of its WHC. The control substrate contained the corresponding amount of deionised water only. Each test vessel was filled with the treated soil and groups of 10 worms were randomly assigned to each treatment group. After approximately 5 minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light but prevented worms from escaping. The test vessels were set up at random in a controlled-environment room.

1. **Dose preparation:**

An exact amount of the test item (10 g) was weighed and dispersed in deionised water to make up a final volume of 1000 mL, and the solution was mixed. This stock solution was diluted with deionised water to prepare further test solutions (serial dilution). Afterwards 60 mL of the test solutions were thoroughly mixed with the artificial soil (750 g wet weight) separately for each replicate by intensive stirring in a laboratory mixer.

1. **Measurements and observations:**

Biological observations:

Individual fresh weight of each adult worm was determined at the start of the test. Observations of behavioural and pathological symptoms (including feeding activity) were made weekly for the test duration. After 4 weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. At the final assessment after 8 weeks, the number of hatched juvenile earthworms in each test vessel was determined.

Physicochemical measurements:

Determination of water content and pH of the artificial soil was done at the start and end of the test.

1. **Statistical analysis:**

The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated.

The EC10, EC20 and EC50 values (number of juveniles) were calculated using the Logit analysis using the maximum likelihood method. Confidence limits (95 %) of the ECx values were computed by normal approximation. For identifying the NOEC values Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, Williams-t-test and Dunnett-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

The statistical analysis was performed with the software ToxRat Professional 3.3.0. (2018).

**II. Results and Discussion**

1. **biological effects**

Mortality rates of 0 – 2.5 % were recorded in the test item treatment groups and 1.3 % in the control. No statistically significant mortality compared to the control was observed at any concentration tested. No pathological symptoms and no further effects on behaviour of the worms were observed. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested.

Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations of 309, 556 and 1000 mg product/kg soil dw.

Table A 74: Summary of effects on mortality and reproduction of the earthworm *Eisenia andrei* following exposure to BAS 743 03 F

| **Treatment group** | **BAS 743 03 F (mg product/kg soil dw)** | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| Mortality (%) day 28 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 0.0 | 2.5 |
| Weight change (%) day 28 | 27.5 | 26.1 | 29.2 | 27.8 | 24.7 | 26.8 | 25.0 | 27.3 | 26.2 |
| Number of juveniles day 56 | 187.8 | 190.8 | 187.8 | 187.0 | 191.5 | 178.3 | 159.0\* | 153.3\* | 146.5\* |
| Reproduction in (%) of control | 100.0 | 101.6 | 100.0 | 99.6 | 102.0 | 94.9 | 84.7 | 81.6 | 78.0 |
|  | **Endpoint (mg product/kg soil dw)** | | | | | | | | |
| 28-day NOEC (mortality) | ≥ 1000 | | | | | | | | |
| 28-day NOEC (biomass) | ≥ 1000 | | | | | | | | |
| 56-day NOEC (reproduction) | 171 | | | | | | | | |
| 28-day LC50 (mortality)1 | > 1000 | | | | | | | | |
| 56-day EC10 (reproduction)2 | n.r. | | | | | | | | |
| 56-day EC20 (reproduction)2 | n.r. | | | | | | | | |
| 56-day EC50 (reproduction)2 | n.d. | | | | | | | | |

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

n.r.: values considered not reliable

\*: statistically significantly different from the control (Williams-t-test for reproduction, α = 0.05, one-sided smaller)

1 based on estimation of the data

2 based on Logit analysis.

In a separate study (BioChem project No. 22 48 TEC 0003, reported 28 Jan 2022), the reference item Maypon Flow (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of earthworms. The number of juveniles was reduced by 51.6 and 99.9 % at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 130.5 and 0.3) after 8 weeks of test duration when compared to control (mean number of juveniles = 269.8). The results of the reference test demonstrated the sensitivity of the test system.

**B. VALIDITY CRITERIA**

All validity criteria were met.

Table A 75: Validity criteria

| **Validity criteria according to OECD 222 (2016)** | **Obtained in this study** |
| --- | --- |
| In the control(s), adult mortality after 4 weeks should be ≤10% | 1.3 % |
| In the control(s), number of juveniles per replicate should be ≥30 | 162 – 220 |
| In the control(s), coefficient of variation of reproduction should be ≤30% | 12.3% |

**C. DEFICIENCIES**

There were no deviations from the study plan.

**III. Conclusion**

In a 56-day earthworm *Eisenia andrei* reproduction study with BAS 743 03 F, no adverse effects on survival and biomass development could be determined at all concentrations tested up to and including 1000 mg product/kg soil dw. The NOEC for mortality and biomass was determined to be ≥1000 mg product/kg soil dw. The NOEC for reproduction was determined to be 171 mg product/kg soil dw. The EC10 and EC20 values for reproduction were considered not reliable and the EC50 value could not be determined but it can be concluded that it is > 1000 mg product/kg soil dw. All validity criteria for the study were met.

* + - * 1. Study 3

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Data point: | CP 10.4.1.1/03 |
| Report | Sublethal toxicity of BAS 650 00 F to the earthworm *Eisenia fetida* in artificial soil with 5% peat  Friedrich, S., 2007  Report No: EU-250291, EU-07 10 48 045 S, EU-66 39 22  XXXX Doc ID: 2007/1037733 |
| Guideline(s): | OECD 222 |
| Deviations: | No |
| GLP: | Yes, (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany) |
| Acceptability: | Yes |

**Executive Summary**

In a chronic toxicity study, adults of *Eisenia fetida* (Annelida: Oligochaeta) were exposed to BAS 650 00 F. The test item was incorporated into artificial soil at concentrations of 6.7, 13.4, 26.9, 53.7 and 107.4 mg BAS 650 00 F/kg dry soil. For the control treatment, the soil was left untreated. The artificial test soil had an organic content of 5% (peat). Assessment of adult earthworm mortality, body weight and feeding activity was carried out after 28 days and reproduction (number of juveniles) was assessed after 56 days.

No mortality was observed in any of the treatment groups and the control. No statistically significant effects on body weight change were observed at any of the test item rates. In the control, a mean number of 130.5 juveniles was counted. In the treatment groups, mean numbers of juveniles between 123.3 and 142.8 were counted. This is corresponding to reproduction rates relative to the control between 94.4% and 109.4%. These differences were not statistically significant compared to the control. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control.

In a 56-day reproduction study with BAS 650 00 Fon earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, reproduction and feeding activity was 107.4 mg BAS 650 00 F/kg dry soil.

**II. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 650 00 F |
| **Lot/Batch:** | 41022 |
| **Active substance content:** | Ametoctradin (BAS 650 F, Reg. No. 4 993 353), 204.4 g/L (nominal: 200.0 g/L) |
| **Density:** | 1.049 g/cm3 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Benlate® (benomyl, 500g/kg nominal). The effects of the toxic standard were investigated in a separate study. |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test organism:** | Earthworm (*Eisenia fetida*) |
| **Age/life stage:** | Adults approx. 3 months old |
| **Body weight:** | 260 – 449 mg |
| **Source:** | In-house culture, originally obtained from W. Neudorff GmbH KG |
| 1. **Environmental conditions** |  |
| **Test substrate:** | Artificial soil according to OECD 222: 5% sphagnum peat |
| **Temperature:** | 18 – 21°C |
| **pH:** | 6.3 – 6.4 at test initiation,  6.2 – 6.3 at test termination |
| **Water content:** | 58.3% - 59.0% of the maximum water holding capacity (WHC) at test initiation and 56.9% - 58.0% of WHC at test termination |
| **Photoperiod:** | 16 h light : 8 h dark (580 lux) |

1. **Test concentrations:**

The study comprised 6 treatment groups (5 test item concentrations and a control). The test concentrations were 6.7, 13.4, 26.9, 53.9 and 107.4 mg BAS 650 00 F/kg dry soil (nominal).

1. **Measurements and observations:**

Mortality, feeding activity, weight change and reproduction rate.

1. **Statistical analysis:**

Descriptive statistics; Dunnett test for weight change and reproduction data (α = 0.05).

**II. Results and Discussion**

**C. biological effects**

No mortality was observed in any of the treatment groups and the control. No statistically significant effects on body weight change were observed at any of the test item rates (Dunnett-test, α = 0.05). In the control, a mean number of 130.5 juveniles was counted. In the treatment groups, mean numbers of juveniles between 123.3 and 142.8 were counted. This is corresponding to reproduction rates relative to the control between 94.4% and 109.4%. These differences were not statistically significant different compared to the control (Dunnett-test, α = 0.05). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control. These results are summarized in the table below.

Table A 76: Effects of BAS 650 00 F on *Eisenia fetida* in a 56-day reproduction study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **BAS 650 00 F [mg/kg dry soil]** | **Control** | **6.7** | **13.4** | **26.9** | **53.7** | **107.4** |
| Mortality (28 d) [%] | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Weight change (28 d) [%] | 43.9 | 43.9 | 46.6 | 42.0 | 45.5 | 39.0 |
| Number of juveniles (56 d) | 130.5 | 141.8 | 142.8 | 123.3 | 131.5 | 125.3 |
| Reproduction in [%] of control (56 d) | 100 | 108.6 | 109.4 | 94.4 | 100.8 | 96.0 |
|  | **Endpoints [mg/kg dry soil]** | | | | | |
| NOEC (28 d) | ≥ 107.4 | | | | | |
| NOEC (56 d) | ≥ 107.4 | | | | | |
| EC50 | ≥ 107.4 | | | | | |

**D. VALIDITY CRITERIA**

All validity criteria were met (Table A 77).

Table A 77: Validity criteria

| **Validity criteria according to OECD 222 (2016)** | **Obtained in this study** |
| --- | --- |
| In the control(s), adult mortality after 4 weeks should be ≤10% | 0% |
| In the control(s), number of juveniles per replicate should be ≥30 | 130.5 |
| In the control(s), coefficient of variation of reproduction should be ≤30% | 21% |

**E. DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

In a 56-day reproduction study with BAS 650 00 F on earthworms (Eisenia fetida), the NOEC for mortality, biomass, reproduction and feeding activity was 107.4 mg BAS 650 00 F/kg dry soil.

* + - 1. KCP 10.4.1.2 Earthworms - field studies

Further studies were not triggered.

* + 1. KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)
       1. KCP 10.4.2.1 Species level testing
          1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 232 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.4.2.1/01 |
| Report | Effects of BAS 743 02 F on the reproduction of the collembolan *Folsomia candida*  Friedrich, S., 2022b  XXXX Study ID: 933752\_11  XXXX Doc ID: 2022/2033721 |
| Guidelines: | OECD 232 (2016) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

A study to determine the effects of BAS 743 02 F on the reproduction of the collembolan *Folsomia candida* was performed over 28 days in the laboratory in artificial soil. The test comprised nine treatment groups; eight nominal test item concentrations between 16.3 and 1000 mg product/kg soil dry weight (dw) and an untreated control. Four replicates were used for the test item treatments and eight replicates were used for the control. Each replicate contained 10 springtails. Assessments of mortality, reproduction and behaviour were carried out 28 days after treatment.

No statistically significant effect on parental mortality was found for any concentration tested. Statistically significant effects on the number of juveniles compared to the control group were recorded at a concentration of 1000 mg product/kg soil dw. The LC50 was determined to be >1000 mg product/kg soil dw, the highest tested concentration. The NOEC for mortality was determined to be ≥ 1000 mg product/kg soil dw. The NOEC for reproduction was determined to be 556 mg product/kg soil dw. The EC10, EC20 and EC50 valuesfor reproduction were determined to be 513, > 1000 and > 1000 mg product/kg soil dw, respectively.

**I. MATERIALS AND METHODS**

1. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Description:** | Soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Density:** | 1.080 g/cm3 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Boric acid, tested in a separate study |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Collembola (*Folsomia candida*) |
| **Age/life stage:** | 9 - 12 days at test start (juveniles) |
| **Source:** | In-house culture, originally purchased from Biologische Bundesanstalt, Berlin-Dahlem, Germany |
| **Diet:** | 2 mg dry yeast per test vessel at the start of the test and again on day 14. |
| 1. **Test units:** | Glass containers approx. 150 mL, each containing  30 g soil dry weight and covered with a lid |
| 1. **Environmental conditions** |  |
| **Test soil:** | Artificial soil according to OECD 232: 5% sphagnum peat, 20% kaolin clay, 0.3% calcium carbonate, 74.7% industrial quartz sand and deionised water |
| **Temperature:** | 19.1 – 21.1 °C |
| **pH:** | Test start: 5.98 – 6.05  Test end: 5.84 – 5.87 |
| **WHCmax:** | 42.6 g/100g soil dw |
| **Water content:** | Test start: 58.5 – 58.7 % of the WHCmax  Test end: 56.6 – 57.5 % of the WHCmax |
| **Photoperiod:** | 16 h light : 8 h dark (590 lux) |

1. **Test organism and treatment:**

The study comprised nine treatment groups; eight test item concentrations and an untreated control. The test item concentrations and their respective active substance contents are summarised in Table A 78. Four replicates were used per test item treatment group and eight replicates were used for the control. Each replicate contained 10 springtails.

Table A 78: Active substance content of the test item at each test concentration

|  |  |  |
| --- | --- | --- |
| **BAS 743 02 F (mg/kg soil dw)** | **Propamocarb (mg a.s./kg soil dw)\*** | **Ametoctradin (BAS 650 F) (mg a.s./kg soil dw)\*** |
| 16.3 | 6.53 | 2.07 |
| 29.4 | 11.8 | 3.73 |
| 52.9 | 21.2 | 6.7 |
| 95.3 | 38.1 | 12.1 |
| 171 | 68.6 | 21.8 |
| 309 | 123.5 | 39.2 |
| 556 | 222.2 | 70.5 |
| 1000 | 400.0 | 127.0 |

\* The amounts of Propamocarb and BAS 650 F were calculated based on the nominal contents a.s. The density (1.080 g/cm³) was taken into account.

Collembola of a uniform age were obtained by transferring egg clusters from the breeding containers to new containers with fresh breeding substrate 12 days prior to the test. After 72 hours, these egg clusters were removed from the containers and the juveniles that had hatched during the preceding 72 hours were fed with granulated dry yeast.

After a further 9 days, the collembola were collected for use in the test. The test item was mixed into the soil prior to addition of the collembola. The collembola were then introduced to each test vessel using an aspirator. The test vessels were placed in a random order and randomly re-positioned once per week. The test containers were tightly covered with a lid and briefly opened twice per week for aeration.

1. **Dose preparation:**

An exact weighed amount of the test item was dispersed in deionised water to make a stock solution without the addition of solubility mediators, immediately prior to application. This stock solution was diluted with deionised water to prepare further test solutions (serial dilution). Afterwards the test solutions were thoroughly mixed with the artificial soil separately for each treatment group by intensive stirring in a laboratory mixer.

1. **Measurements and observations:**

Biological observations:

After 4 weeks, the parental and juvenile collembolans in the test and control vessels were counted. The test substrate of each replicate was poured into an individual container (approx. 200 mL volume) and the test organisms were floated off the substrate with the addition of water. After gentle stirring, the number of parental and juvenile collembolans floating on the surface was determined. Missing parental collembolans were assumed dead. Surviving adults and juveniles were counted using an automated counting technique based on a video camera connected to a digital image storage and analysis system (LemnaTec Scanalyzer). Observations of any physiological or pathological symptoms or distinct changes in behaviour were also recorded.

Physicochemical measurements:

The pH and water content of the test substrate were determined at the start and end of the test. The water content was checked weekly by reweighing the additional test vessels. Water loss was compensated if exceeding 2 % of the initial water content.

1. **Statistical analysis:**

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018). Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, Williams-t-test and logit analysis were used to compare the control with the independent test item groups.

**II. Results and Discussion**

1. **biological effects**

No statistically significant effect on parental mortality was found for any concentration tested. Mortality rates between 0.0 % - 5.0 % were recorded in the test item treatment groups. In the control, the mortality rate was 2.5 %. Statistically significant effects on the number of juveniles compared to the control group were recorded at a concentration of 1000 mg product/kg soil dw. No differences were observed in the behaviour of the collembolans between the control group and the test item treatment groups.

Results are shown in Table A 79.

Table A 79: Summary of effects on mortality and reproduction of the collembolan *Folsomia candida* following 28 days exposure to BAS 743 02 F

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment group** | **BAS 743 02 F (mg product/kg soil dw)** | | | | | | | | |
| **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| Mortality (%) | 2.5 | 2.5 | 0.0 | 2.5 | 2.5 | 2.5 | 0.0 | 2.5 | 5.0 |
| Mean number of juveniles | 1094 | 1063 | 1084 | 1095 | 1067 | 1048 | 1070 | 1090 | 902\* |
| Reproduction in (%) of control | 100 | 97.2 | 99.1 | 100.1 | 97.5 | 95.8 | 97.8 | 99.6 | 82.5 |
|  | **Endpoint (mg product/kg soil dw)** | | | | | | | | |
| NOEC (mortality) | ≥ 1000 | | | | | | | | |
| NOEC (reproduction) | 556 | | | | | | | | |
| LC50 (mortality)1 | > 1000 | | | | | | | | |
| EC10 (reproduction)2 | 513 (225 – 1292) | | | | | | | | |
| EC20 (reproduction)2 | > 1000 | | | | | | | | |
| EC50 (reproduction)2 | > 1000 | | | | | | | | |

\* Statistically significantly different compared to the control (α = 0.05, one-sided greater; Williams-t-test for reproduction; α = 0.05, one-sided smaller)

Calculations were performed with unrounded values

1 Due to negligible effects and lacking dose response the value was estimated to be above the highest test concentration

2 Dased on Logit analysis

In a separate study (BioChem project No. 22 48 TCC 0024, dated 05 Sep 2022), the EC50 (reproduction) of the reference item boric acid was calculated to be 106 mg/kg soil dw. The results of the reference test demonstrate the sensitivity of the test system.

1. **VALIDITY CRITERIA**

All validity criteria were met (Table A 80).

Table A 80: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 232 (2016)** | **Obtained in this study** |
| In the control(s), mean adult mortality in the control must be ≤20% | 2.5% |
| In the control(s), mean number of juveniles per test vessel in the controls must be ≥100 | Average of 1094/vessel |
| In the control(s), coefficient of variation for the mean number of juveniles must be <30% | 7.7% |

1. **DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

In a 28-day *Folsomia candida* reproduction study, in which Collembolans were exposed to BAS 743 02 F, the LC50 was determined to be > 1000 mg product/kg soil dw, the highest tested concentration. The NOEC for mortality was determined to be ≥ 1000 mg test item/kg soil dw and the NOEC for reproduction was determined to be 556 mg product/kg soil dw. The EC10, EC20 and EC50 values for reproduction were calculated to be 513, > 1000 and > 1000 mg product/kg soil dw, respectively.

* + - * 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 226 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.4.2.1/02 |
| Report | Effects of BAS 743 02 F on the reproduction of the predatory mite *Hypoaspis aculeifer*  Schulz L., 2023a  XXXX Study ID: 933752\_12  XXXX Doc ID: 2022/2033721 |
| Guideline(s): | OECD 226 (2016) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The effects of BAS 743 02 F on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a laboratory study over 14 days. Eight test concentrations, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg product/kg soil dry weight (dw), were incorporated into the soil with four replicates per concentration. An untreated control with eight replicates was included. Each replicate treatment contained 10 adult female mites. The reference item was tested in a separate study. Assessment of mortality and reproduction (number of juveniles) was carried out after 14 days.

In the14-day *Hypoaspis aculeifer* reproduction study with BAS 743 02 F, the LC50, EC10, EC20 and EC50 values could not be calculated due to the non-existing dose response and negligible effects, but it can be concluded that these values are > 1000 mg product/kg soil dw, respectively. The NOEC for mortality and for reproduction were determined to be ≥ 1000 mg product/kg soil dw, the highest concentration tested.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Lot/Batch:** | FRE-002224 |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Density:** | 1.080 g/cm3 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Dimethoate (EC 400 g/L, nominal). The effects of the reference item were investigated in a separate study. |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | *Hypoaspis aculeifer* (Canestrini) |
| **Age/life stage:** | Adult mites with an age difference of 2 days |
| **Diet:** | *Tyrophagus putrescentiae* (Schrank) at test start and *ad libitum* during the test |
| **Source:** | In-house culture of the test facility |
| 1. **Environmental conditions** |  |
| **Test soil:** | Artificial soil according to OECD 226: 5% sphagnum peat, 20% kaolin clay, 0.225% calcium carbonate, 74.775% industrial quartz sand (predominantly fine) |
| **Temperature:** | 20.4 - 21.5 °C |
| **pH:** | pH 6.3 - 6.5 at test initiation,  pH 5.8 - 5.9 at test termination |
| **Water content:** | At test initiation: 18.62 - 19.15 g/100 g soil d.w. (equivalent to 48.56 - 49.95 % of WHC)  At test termination: 18.30 - 18.75 g/100 g soil d.w (equivalent to 47.74 - 48.90 % of WHC) |
| **Photoperiod:** | 16 h light: 8 h dark; light intensity: 609 lx |

1. **Test organism and treatment:**

The study comprised nine treatment groups; eight test item concentrations and an untreated control. The test item concentrations and their respective active substance contents are summarised in Table A 81. Four replicates were used per test item treatment group and eight replicates were used in the control. Each replicate contained 10 adult female soil mites.

Table A 81 Active substance content of the test item at each test concentration

|  |  |  |
| --- | --- | --- |
| **BAS 743 02 F (mg/kg soil dw)** | **Propamocarb (mg a.s./kg soil dw)\*** | **Ametoctradin / BAS 650 F (mg a.s./kg soil dw)\*** |
| 16.3 | 6.5 | 2.07 |
| 29.4 | 11.8 | 3.73 |
| 52.9 | 21.2 | 6.7 |
| 95.3 | 38.1 | 12.1 |
| 171 | 68.6 | 21.8 |
| 309 | 123.5 | 39.2 |
| 556 | 222.2 | 70.5 |
| 1000 | 400.0 | 127.0 |

\* The amounts of Propamocarb and BAS 650 F were calculated based on the nominal contents a.s. The density (1.080 g/cm³) was taken into account.

Adult females were transferred to clean rearing vessels and incubated at approximately 20°C for 2 days. The adult mites were then removed and the rearing vessels, now containing eggs, were incubated for a further 28 days at the same temperature until the eggs had developed into adult mites. Within this incubation time the laid eggs developed to adult mites of similar age. At day 30, the mites of the synchronised culture were ready for the test.

At the start of the test (within 2 hours after treatment of the soil), adult females of the synchronised culture were transferred to the prepared test vessels which contained untreated or test item treated artificial soil. The vessels were briefly opened every 2-3 days for aeration and feeding during the test.

1. **Dose preparation:**

An exact weighed amount of the test item was mixed with deionised water to make a stock solution, without addition of solubility mediators, immediately before application. This stock solution was stepwise diluted with deionised water to prepare seven further test solutions, Afterwards the test solutions were thoroughly mixed with the artificial soil by means of a hand stirrer.

1. **Measurements and observations:**

Biological observations:

On day 14, surviving mites and juveniles were extracted from each vessel using a MacFadyen high gradient extractor (heat/light extraction method). The duration of extraction was 48 hours. During this time, adult and juvenile mites moved down through the soil substrate away from the heat source, until they fell from the substrate into the funnel / fixing liquid. After extraction was complete, all juveniles and adults present in the fixing liquid were counted. Missing mites were assumed dead. Mortality and reproduction in each treatment group were determined.

Physicochemical measurements:

Water content and pH were measured at the start and end of the test. Water content was maintained throughout the test by reweighing the additional test vessels. Compensation of water loss was necessary.

1. **Statistical analysis:**

The Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (α = 0.05, one-sided greater) was used for mortality and the Dunnett’s Multiple t-test Procedure (α = 0.05, one-sided smaller), was used for reproduction to compare the control with the independent test item groups.

No ECx calculations were performed because of negligible effects and missing dose response.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018).

**II. Results and Discussion**

1. **biological effects**

The mortality was between 0% and 7.5% in the test item treatment groups, compared to 5.0% in the control. The mortality observed was not statistically significantly different compared to the control. Reproduction rates in the 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg product/kg soil dw groups were 305.3, 294.0, 299.0, 308.5, 308.3, 297.8, 290.5 and 289.3 juveniles, respectively. The mean reproduction in the control reached 292.0 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations. No behavioral abnormalities or differences in morphology of the mites between the test item treated groups and the control were observed. The results are summarized in Table A 82.

Table A 82: Effects of BAS 743 02 F on mortality and reproduction of predatory mites *(Hypoaspis aculeifer)* in a 14-day study

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment group** | **BAS 743 02 F (mg product/kg soil dw)** | | | | | | | | |
| **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171** | **309** | **556** | **1000** |
| Mortality (%) | 5.0 | 2.5 | 2.5 | 5.0 | 7.5 | 0.0 | 5.0 | 5.0 | 2.5 |
| Mean number of juveniles | 292.0 | 305.3 | 294.0 | 299.0 | 308.5 | 308.3 | 297.8 | 290.5 | 289.3 |
| Reproduction in (%) of control | 100 | 105 | 101 | 102 | 106 | 106 | 102 | 99 | 99 |
|  | **Endpoint (mg product/kg soil dw)** | | | | | | | | |
| NOEC (mortality) | ≥ 1000 | | | | | | | | |
| NOEC (reproduction) | ≥ 1000 | | | | | | | | |
| LC50 (mortality)1 | > 1000 | | | | | | | | |
| EC10 (reproduction)1 | ≥ 1000 | | | | | | | | |
| EC20 (reproduction)1 | > 1000 | | | | | | | | |
| EC50 (reproduction)1 | > 1000 | | | | | | | | |

1) Based on estimation.

The calculations were performed with unrounded values.

In a separate study (BioChem project No. 22 48 THC 0015, dated: 06 May 2022), the EC50 (reproduction) of the reference item Dimethoate 400 EC (400 g/L, nominal) was calculated to be 4.93 mg a.s./kg soil dw The results of the reference test demonstrate the sensitivity of the test system.

1. **VALIDITY CRITERIA**

All validity criteria were met (Table A 83).

Table A 83: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 226 (2016)** | **Obtained in this study** |
| In the control(s), mean mortality of adult females must be ≤20% | 5.0% |
| In the control(s), mean number of juveniles per replicate must be ≥50 | 292.0 |
| In the control(s), the coefficient of variation for the mean number of juveniles per replicate must be ≤30% | 4.0% |

1. **DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 743 02 F, the LC50, EC10, EC20 and EC50 values could not be calculated, but it can be concluded that these values are > 1000 mg product/kg soil dw, respectively. The NOEC for mortality and for reproduction were determined to be ≥ 1000 mg product/kg dry soil, the highest concentration tested.

* + - * 1. Study 3

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Data point: | CP 10.4.2.1/03 |
| Report | Effects of BAS 650 00 F on the reproduction of the collembolans *Folsomia candida* in artificial soil with 5% peat  Friedrich, S., 2007  Report No EU-275161, EU-07 10 48 042 S, EU-86 14 33  XXXX Doc ID: 2007/1037734 |
| Guideline(s): | ISO 11267 (1999) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The effects of BAS 650 00 F on survival and reproduction of the collembolan *Folsomia candida* were investigated in a laboratory study over 28 days. The test item was incorporated into the soil at concentrations of 62.5, 125, 250, 500, and 1000 mg BAS 650 00 F/kg dry soil. For the control treatment, the soil was left untreated. The artificial test soil had an organic content of 5% (peat). Assessment of mortality of the adults and reproduction (number of juveniles) was carried out after 28 days.

At the control, a mortality rate of 2% was observed. At the test item concentrations, mortality rates were between 2% and 4%. These mortalities were not statistically significantly different compared to the control.

In the control, a mean of 455.6 juveniles was counted. In the treatment groups, a mean number of juveniles between 359.4 and 482.4 were counted. This is corresponding to a reproduction relative to the control between 79% and 106%. No differences in reproduction were recorded.

In a 28-day reproduction study with BAS 650 00 F on springtails, the NOEC was 1000 mg BAS 650 00 F/kg dry soil. The LC50 and EC50 was determined to be > 1000 mg BAS 650 00 F/kg dry soil.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 650 00 F |
| Lot/Batch: | 41022 |
| Active substance content: | Ametoctradin (BAS 650 F, Reg. No. 4 993 353), 204.4 g/L (nominal: 200.0 g/L) |
| Density: | 1.049 g/cm3 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Betosip (phenmedipham 114 g/L; 15.4% analyzed). The effects of the reference item were investigated in a separate study. |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Collembola (*Folsomia candida*), |
| Age/life stage: | 10 - 12 days old |
| Source: | In-house culture |
| 1. **Environmental conditions** |  |
| Test soil: | Artificial soil according to ISO 11267 (5% peat); |
| Temperature: | 18 – 22°C |
| pH: | pH 6.1– 6.2 at test initiation and  pH 6.2 at test termination |
| Water content: | At study initiation: 58.0% - 58.3% of maximum water holding capacity (WHC) and 57.1% - 57.8% of maximum WHC at test termination. |
| Photoperiod: | 16 h light : 8 h dark (590 lux) |

1. **Test design:**

In a 28-day test, adults of *Folsomia candida* were exposed to BAS 650 00 F incorporated into the soil. In total, 6 treatment groups were set up (5 concentrations of the test item and an untreated control group) with 5 replicates for each treatment group with 10 collembolans per replicate. The artificial soil was treated and filled into glass vessels, before the collembolans were introduced on the top of the soil.

1. **Test concentrations:**

Control, 62.5, 125, 250, 500, and 1000 mg BAS 650 00 F/kg dry soil.

1. **Measurements and observations:**

Collembolans mortality, behavioral effects and reproduction (number of juveniles) were assessed after 28 days.

1. **Statistical analysis:**

Descriptive statistics; Fisher’s Exact test for mortality data and Dunnett’s –Test for reproduction data (α = 0.05).

**II. Results and Discussion**

**C. BIOLOGICAL EFFECTS**

At the control, a mortality rate of 2% was observed. At the test item concentrations, mortality rates were between 2% and 4%. These mortality rates were neither dose related, nor these mortality rates did differ statistically significantly compared to the control (Fisher’s Exact test, α = 0.05).

In the control, a mean of 455.6 juveniles was counted. In the treatment groups, a mean number of juveniles between 359.4 and 482.4 were counted. This is corresponding to a reproduction relative to the control between 79% and 106%. No statistically significant differences on reproduction were recorded (Dunnett’s-Test, α = 0.05). The results are summarized in the table below.

Table A 84: Effects of BAS 650 00 F on *Folsomia candida* in a 28-day reproduction study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **BAS 650 00 F [mg/kg dry soil]** | **Control** | **62.5** | **125** | **250** | **500** | **1000** |
| Mortality (day 28) [%] | 2 | 4 | 2 | 4 | 2 | 2 |
| No. of juveniles (day 28) | 455.6 | 482.4 | 405.2 | 362.4 | 359.4 | 414.8 |
| Reproduction in [%] of control (day 28) | -- | 106 | 89 | 80 | 79 | 91 |
| Coefficient of variation of reproduction (%) | 15.3 | 12.8 | 12.1 | 22.1 | 10.2 | 19.5 |
| **Endpoints** | **[mg BAS 650 00 F/kg dry soil]** | | | | | |
| NOEC | ≥ 1000 | | | | | |
| EC50 | > 1000 | | | | | |
| LC50 | > 1000 | | | | | |

The reference item Betosip had a significant effect on mortality with a LC50 of 221.8 mg /kg dry soil, the EC50 for reproduction was 146.4 mg/kg dry soil. Reproduction was statistically significantly reduced by 100 mg/kg dry soil. The NOEC was 50 mg/kg dry soil.

**D. VALIDITY CRITERIA**

All validity criteria were met (Table A 85).

Table A 85: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 232 (2016)** | **Obtained in this study** |
| In the control(s), mean adult mortality in the control must be ≤20% | 2% |
| In the control(s), mean number of juveniles per test vessel in the controls must be ≥100 | Average of 455.6 |
| In the control(s), coefficient of variation for the mean number of juveniles must be <30% | 15.3% |

**E. DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

In a 28-day reproduction study with BAS 650 00 F on springtails, the NOEC was ≥ 1000 mg BAS 650 00 F/kg dry soil. The LC50 and EC50 was determined to be > 1000 mg BAS 650 00 F/kg dry soil.

* + - * 1. Study 4

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Data point: | CP 10.4.2.1/04 |
| Report | Effects of BAS 650 00 F on the reproduction of the predatory mite *Hypoaspis aculeifer*  Schulz L., 2016  Report No EU-808981  XXXX Doc ID: 2016/1193035 |
| Guideline(s): | OECD 226 (2008) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The effects of BAS 650 00 F on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a laboratory study over 14 days. Eight test concentrations, 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg BAS 650 00 F/kg dry soil were incorporated into the soil with four replicates per concentration. An untreated control with 8 replicates was included. Each replicate treatment contained 10 adult female mites. The reference item was tested in a separate study. Assessment of mortality and reproduction (number of juveniles) was carried out after 14 days.

The mortality was between 0% and 10% in the test item treatment groups, compared to 3.8% in the control. The mortality observed was not statistically significantly different compared to the control. Reproduction rates in the 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg dry soil groups were 297.8, 284.8, 286.3, 282.0, 315.3, 291.0, 322.0 and 293.5 juveniles, respectively. The mean reproduction in the control reached 290.6 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations. No behavioral abnormalities or differences in morphology of the mites between the test item treated groups and the control were observed.

In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 650 00 F, the LC50, EC10, EC20 and EC50 values could not be calculated, but it can be concluded that these values are higher than 1000 mg BAS 650 00 F/kg dry soil, respectively. The NOEC for mortality and for reproduction were determined to be ≥ 1000 mg BAS 650 00 F/kg dry soil, the highest concentration tested.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 650 00 F |
| **Lot/Batch:** | FRE-001377 |
| **Active substance content:** | Ametoctradin (BAS 650 F, Reg. No. 4 993 353), 200.1 g/L (nominal: 200.0 g/L) |
| **Density:** | 1.049 g/cm3 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Dimethoate (EC 400 g/L, nominal). The effects of the reference item were investigated in a separate study. |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | *Hypoaspis aculeifer* (CANESTRINI) |
| **Age/life stage:** | Adult mites with an age difference of 3 days |
| **Diet:** | *Tyrophagus putrescentiae* (SCHRANK) at test start and *ad libitum* during the test |
| **Source:** | In-house culture of the test facility |
| 1. **Environmental conditions** |  |
| **Test soil:** | Artificial soil according to OECD 226: 5% sphagnum peat |
| **Temperature:** | 19.8 - 20.2 °C |
| **pH:** | pH 6.0 - 6.3 at test initiation,  pH 5.9 - 6.0 at test termination |
| **Water content:** | At test initiation 43.71% - 48.39% of maximum water holding capacity (WHC) and 42.80% - 46.94% of maximum WHC at test termination |
| **Photoperiod:** | 16 h light: 8 h dark; light intensity: 521 lx |

1. **Test design:**

14-day chronic laboratory test in treated artificial soil (according to OECD 226). Different concentrations of the test item were homogenously mixed into the soil (5% peat) which was filled in glass vessels before the predatory mites were introduced on top of the soil; 9 treatment groups (eight test item concentrations, deionized water control); with 8 replicates for the control treatment group and 4 replicates for test item treatment groups, each with 10 female mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

1. **Test concentrations:**

Control; 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg BAS 650 00 F/kg dry soil (spacing factor: 1.8).

1. **Measurements and observations:**

Assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

1. **Statistical analysis:**

Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality (α = 0.05, one-sided greater), Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm for reproduction (α = 0.05, one-sided smaller).

**II. Results and Discussion**

**C. BIOLOGICAL EFFECTS**

The mortality was between 0% and 10% in the test item treatment groups, compared to 3.8% in the control. The mortality observed was not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater). Reproduction rates in the 16.3, 29.4, 52.9, 95.3, 171.5, 308.6, 555.6 and 1000 mg test item/kg dry soil groups were 297.8, 284.8, 286.3, 282.0, 315.3, 291.0, 322.0 and 293.5 juveniles, respectively. The mean reproduction in the control reached 290.6 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations (Multiple Sequentially-rejective Welch-t-test after Bonferroni-Holm, α = 0.05, one-sided smaller). No behavioral abnormalities or differences in morphology of the mites between the test item treated groups and the control were observed. The results are summarized in Table A 86.

Table A 86: Effects of BAS 650 00 F on mortality and reproduction of predatory mites *(Hypoaspis aculeifer)* in a 14-day study

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **BAS 650 00 F [mg/kg dry soil]** | **Control** | **16.3** | **29.4** | **52.9** | **95.3** | **171.5** | **308.6** | **555.6** | **1000** |
| Mean mortality (day 28) [%] | 3.8 | 5.0 | 0.0 | 2.5 | 2.5 | 5.0 | 7.5 | 2.5 | 10.0 |
| Mean no. of juveniles (day 28) | 290.6 | 297.8 | 284.8 | 286.3 | 282.0 | 315.3 | 291.0 | 322.0 | 293.5 |
| Reproduction in [%] of control (day 28) | 100 | 102 | 98 | 98 | 97 | 108 | 100 | 111 | 101 |
| Coefficient of variation of the number of juveniles [%] | 7.9 | 17.7 | 3.2 | 10.8 | 6.8 | 12.2 | 3.8 | 11.2 | 14.6 |
| **Endpoints** | **[mg BAS 650 00 F/kg dry soil]** | | | | | | | | |
| NOECmortality, reproduction | ≥ 1000 | | | | | | | | |
| EC10 1) | > 1000 | | | | | | | | |
| EC20 1) | > 1000 | | | | | | | | |
| EC50 1) | > 1000 | | | | | | | | |
| LC50 1) | > 1000 | | | | | | | | |

1) Based on estimation.

In a separate study the EC50 (reproduction) of the reference item dimethoate (EC 400 g/L, nominal) was calculated to be 3.1 mg a.s./kg dry soil.

**D. VALIDITY CRITERIA**

All validity criteria were met (Table A 87).

Table A 87: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 226 (2016)** | **Obtained in this study** |
| In the control(s), mean mortality of adult females must be ≤20% | 3.8% |
| In the control(s), mean number of juveniles per replicate must be ≥50 | 290.6 |
| In the control(s), the coefficient of variation for the mean number of juveniles per replicate must be ≤30% | 7.9% |

**E. DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 650 00 F, the LC50, EC10, EC20 and EC50 values could not be calculated, but it can be concluded that these values are higher than 1000 mg BAS 650 00 F/kg dry soil, respectively. The NOEC for mortality and for reproduction were determined to be ≥ 1000 mg BAS 650 00 F/kg dry soil, the highest concentration tested.

* + - * 1. Study 5

XXXX have a Letter of Access allowing them to rely on this study.

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | None |
| Report | Propamocarb hydrochloride SL 722 G: Effects on the reproduction of the collembolan *Folsomia candida*  Friedrich, S., 2014  Biochem Project No.: 14 10 48 247 S |
| Guideline(s): | OECD 232 (2009), ISO 11267 (1999) |
| Deviations: | No |
| GLP: | yes | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

A study to determine the effects of Propamocarb hydrochloride SL 722 G (active substances propamocarb hydrochloride) on the reproduction of the collembolan *Folsomia candida* was performed over 28 days in the laboratory in artificial soil. The test comprised 6 treatment groups; 5 nominal test item concentrations of 100, 178, 316, 562 and 1000 mg test item/kg soil dry weight and an untreated control. 4 replicates were used for the test item treatments and 8 replicates were used for the control. Each replicate contained 10 collembola. After 28 days, parental mortality and total juveniles per treatment were determined.

The 28-day LC50 and the EC10, EC20 and EC50 values for reproduction could not be calculated due to an absence of statistically significant effects. It was determined that these values were >1000 mg test item/kg soil dw, the highest concentration tested. The NOEC for mortality and reproduction was determined to be ≥1000 mg test item/kg soil dw and the LOEC for mortality and reproduction was determined to be >1000 mg test item/kg soil dw.

**II. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | Propamocarb hydrochloride SL 722 G |
| **Description:** | clear colourless liquid |
| **Lot/Batch:** | NP65DX4580 |
| **Active substance content:** | Propamocarb hydrochloride 722 g/L (nominal),  67.7 % w/w, 729.1 g/L (analysed) |
| **Density:** | 1.077 g/mL |
| **Storage conditions:** | 25 ± 5 °C, +2 °C to +30 °C are also acceptable |
| **Stability:** | Expiry date: 07.07.2015 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Boric acid, tested in a separate study |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | Collembola (*Folsomia candida* WILLEM), |
| **Age/life stage:** | 9 - 12 days at test start (juveniles) |
| **Source:** | In-house culture, originally purchased from Biologische Bundesanstalt, Berlin-Dahlem, Germany |
| **Diet:** | 2 mg granulated dry yeast per test vessel at the start of the test and again on day 14. |
| 1. **Test units:** | Glass containers approx. 150 mL, each containing  30 g soil wet weight and covered with a lid |
| 1. **Environmental conditions** |  |
| **Test soil:** | Artificial soil according to OECD 232: 5% sphagnum peat, 20% kaolin clay, 0.3% calcium carbonate, 74.7% industrial quartz sand and deionised water |
| **Temperature:** | 18.0 – 20.7 °C |
| **pH:** | Test start: 6.04 – 6.08  Test end: 5.77 – 5.82 |
| **WHCmax:** | 42.8 g/100g soil dw |
| **Water content:** | Test start: 25.0 – 25.1% (equivalent to 58.4 – 58.6% of WHC)  Test end: 24.4 – 24.8% (equivalent to 57.0 – 57.9% of WHC) |
| **Photoperiod:** | 16 h light : 8 h dark (460 lux) |

1. **Test organism and treatment:**

The test comprised 6 treatment groups; 5 nominal test item concentrations of 100, 178, 316, 562 and 1000 mg test item/kg soil dry weight and an untreated control. 4 replicates were used for the test item treatments and 8 replicates were used for the control. Each replicate contained 10 collembola.

Collembola of a uniform age were obtained by transferring egg clusters from the breeding containers to new containers with fresh breeding substrate 12 days prior to the test. After 72 hours, these egg clusters were removed from the containers and the juveniles that had hatched during the preceding 72 hours were fed with granulated dry yeast.

After a further 9 days, the collembola were collected for use in the test. The test item was mixed into the soil prior to addition of the collembola. The collembola were then introduced to each test vessel using an exhauster. The test vessels were placed in a random order and randomly re-positioned once per week. The test containers were tightly covered with a lid and briefly opened twice per week for aeration.

1. **Dose preparation:**

An exactly weighed amount of the test item was dissolved in deionised water to make a stock solution, without addition of solubility mediators, immediately before application. This stock solution was diluted with deionised water such that each solution contained the amount of test item in 25 mL required to dose 250 g dry weight equivalent of artificial soil. Each test item solution (25 mL) was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC.

1. **Measurements and observations:**

After 4 weeks, the parental and juvenile collembolans in the test and control vessels were counted. Observations of any physiological or pathological symptoms or distinct changes in behaviour were also recorded.

The test substrate of each replicate was poured into an individual container (approx. 150 - 200 mL volume) and the test organisms were floated off the substrate with the addition of water. After gentle stirring, the number of parental and juvenile collembolans floating on the surface was determined. Missing parental collembolans were assumed dead. Surviving adults and juveniles were counted using an automated counting technique based on a video camera connected to a digital image storage and analysis system (LemnaTec Scanalyzer).

The pH and water content of the test substrate were determined at the start and end of the test.

1. **Statistical analysis:**

The statistical analysis was performed with the software ToxRat Professional 2.10.06 (Ratte 2010). Fisher’s Exact Binomial Test and Williams-t-test were used to compare the control with the independent test item group

**II. Results and Discussion**

**C. BIOLOGICAL EFFECTS**

Mortality rates of 0 % - 2.5 % were recorded in the test item treatment groups. 2.5 % parental mortality was observed in the control. No statistically significant effect (Fisher`s Exact Binomial Test, α = 0.05, one-sided greater) on parental mortality was found for any concentration tested. Mortality rates are summarised in Table A 73. No effects on behaviour of the collembolans were observed during the test. The NOEC for the mortality of parental collembolans was determined to be ≥ 1000 mg test item/kg soil d.w.

The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 497 in the control and 520, 500, 507, 488 and 517 at concentrations of 100, 178, 316, 562 and 1000 mg test item/kg soil d.w., respectively. The mean number of juveniles counted 28 days after introduction of the parental collembolans are presented in Table A 88 No statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles were found for any concentration tested. The no-observed-effect-concentration (NOEC) for reproduction was determined to be ≥ 1000 mg test item/kg soil d.w.

Table A 88: Summary of effects on mortality and reproduction of the collembolan *Folsomia candida* following 28 days exposure to Propamocarb hydrochloride SL 722 G in soil

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Propamocarb hydrochloride SL 722 G (mg test item/kg soil dw)** | | | | | |
| **Control** | **100** | **178** | **316** | **562** | **1000** |
| Mortality (%) day 28 | 2.5 | 2.5 | 0.0 | 2.5 | 2.5 | 2.5 |
| Mean number of juveniles (day 28) | 497 | 520 | 500 | 507 | 488 | 517 |
| Reproduction in (%) of control | 100 | 105 | 101 | 102 | 98 | 104 |
|  | **Endpoint (mg test item/kg soil dw)** | | | | | |
| NOEC (mortality/ reproduction) | ≥1000 | | | | | |
| LOEC (mortality/ reproduction) | >1000 | | | | | |

Not statistically significantly different compared to the control (Fisher’s Exact Binomial Test with Bonferroni Correctionfor mortality, α = 0.05, one-sided greater; Williams-t-test;  = 0.05, one-sided smaller for reproduction, α = 0.05, one-sided smaller)

Calculations were performed with unrounded values

To verify the sensitivity of the test system the reference item boric acid is routinely tested at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dry weight. The collembolans of the reference test were from the same source culture as those used in the definitive test. In the most recent study (BioChem project No. R 14 10 48 003 S, dated July 30, 2014) the EC50 was determined to be 104 mg/kg soil dry weight. The LC50 was determined to be 181 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight, respectively. The EC50 value for the reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2009). The EC50 therefore showed that the test system was sensitive.

**D. VALIDITY CRITERIA**

All validity criteria were met (Table A 89).

Table A 89: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 232 (2016)** | **Obtained in this study** |
| In the control(s), mean adult mortality in the control must be ≤20% | 2.5% |
| In the control(s), mean number of juveniles per test vessel in the controls must be ≥100 | Average of 497/vessel |
| In the control(s), coefficient of variation for the mean number of juveniles must be <30% | 8.9% |

**E. DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

A study to determine the effects of Propamocarb hydrochloride SL 722 G, (active substances propamocarb hydrochloride) on the reproduction of the collembolan *Folsomia candida* was performed over 28 days in the laboratory in artificial soil. After 28 days, parental mortality and total juveniles per treatment were determined.

The test item Propamocarb hydrochloride SL 722 G showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil up to and including 1000 mg test item/kg d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg d.w.

* + - * 1. Study 4

XXXX have a Letter of Access allowing them to rely on this study

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | None |
| Report | Propamocarb-hydrochloride SL 722 G: Effects on the reproduction of the predatory mite Hypoaspis aculeifer  Schulz L., 2014  BioChem project No.: 14 10 48 248 S  Reference No.: EBPRN020 |
| Guideline(s): | OECD 226 (2008) |
| Deviations: | No |
| GLP: | yes | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of Propamocarb-hydrochloride SL 722 G on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a laboratory study over 14 days. A limit test concentrations of 1000 mg test item/kg dry soil were incorporated into the soil with 8 replicates per concentration. An untreated control with 8 replicates was included. Each replicate treatment contained 10 adult female mites. The reference item was tested in a separate study. Assessment of mortality and reproduction (number of juveniles) was carried out after 14 days.

The mortality was between 1.3% in the test item treatment groups, compared to 5.0% in the control. The mortality observed was not statistically significantly different compared to the control. Reproduction rates in the 1000 mg test item/kg dry soil groups was 311.5 juveniles. The mean reproduction in the control reached 305.5 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations. No behavioral abnormalities or differences in morphology of the mites between the test item treated groups and the control were observed.

In a 14-day *Hypoaspis aculeifer* reproduction study with Propamocarb-hydrochloride SL 722 G, the LC50, EC10, EC20 and EC50 values could not be calculated, but it can be concluded that these values are higher than 1000 mg test item/kg dry soil, respectively. The NOEC for mortality and for reproduction were determined to be ≥ 1000 mg test item/kg dry soil.

**I. MATERIALS AND METHODS**

**A. MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | Propamocarb-hydrochloride SL 722 G |
| **Lot/Batch:** | NP65DX4580 |
| **Active substance content:** | Propamocarb hydrochloride 722 g/L (nominal),  67.7 % w/w, 729.1 g/L (analysed) |
| **Density:** | 1.077 g/mL |
| **Storage conditions:** | 25 ± 5 °C, +2 °C to +30 °C are also acceptable |
| **Stability** | 07.07.2015 |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Dimethoate. The effects of the reference item were investigated in a separate study. |

**B. study design and methods**

|  |  |
| --- | --- |
| 1. **Test species:** | *Hypoaspis aculeifer* (CANESTRINI) |
| **Age/life stage:** | Adult mites with an age difference of 3 days |
| **Diet:** | *Tyrophagus putrescentiae* (SCHRANK) at test start and *ad libitum* during the test |
| **Source:** | In-house culture, originally obtained from “Bayer CropScience AG” Monheim |
| 1. **Environmental conditions** |  |
| **Test vessel:** | 100 mL SCHOTT-bottle with screw cap (inside dimensions: 4 cm in diameter, 11 cm high) |
| **Test soil:** | Artificial soil according to OECD 226: 5% sphagnum peat |
| **Temperature:** | 19.7 - 21.2 °C |
| **pH:** | test start: 5.7  test termination: 5.5 - 5.6 |
| **Max. water holding capacity:** | 36.09 g/100 g soil d.w. |
| **Water content:** | test initiation: 17.81 - 18.46 (equivalent to 46.36 - 51.16 % of WHC)  test termination: 18.08 - 18.38 (equivalent to 50.10 - 50.92 % of WHC) |
| **Photoperiod:** | 16 h light: 8 h dark; light intensity: 518 lx |

1. **Test design:**

14-day chronic laboratory test in treated artificial soil (according to OECD 226). A limit concentration of 1000 mg/kg dry soil was homogenously mixed into the soil (5% peat) which was filled in glass vessels before the predatory mites were introduced on top of the soil; 8 replicates per concentration. An untreated control with 8 replicates was included. Each replicate treatment contained 10 adult female mites. Assessment of mortality and reproduction (number of juveniles) was carried out after 14 days.

1. **Test concentrations:**

Control; 1000 mg test item/kg dry soil (limit test). An exactly weighed amount of the test item was mixed in a volumetric flask with deionised water, without addition of solubility mediators, immediately before application. The test item solution was then thoroughly mixed with the prepared artificial soil by means of a hand stirrer. The preparation of the test substrate was performed in the following order: first the untreated control and thereafter the test item treated group

1. **Measurements and observations:**

Assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

On day 14 following application of the test item and introduction of the test organisms, surviving mites and juveniles of *Hypoaspis aculeifer* were extracted from each test replicate using a MacFadyen high-gradient

extractor (heat/light extraction method). Following extraction, all juveniles and adults present in the fixing liquid were counted. Any adult mites not found after extraction were recorded as dead.

The water content of the soil substrate in the test vessels was determined at test start (after application) and at day 14 after application and was maintained throughout the test by reweighing the additional test vessels. Compensation of water loss was not necessary. The vessels were briefly opened every 2 - 3 days for aeration and feeding.

1. **Statistical analysis:**

The statistical analysis was performed with the software ToxRat Professional 2.10.05 (Ratte 2010). Fisher´s Exact Binomial test and the Student-t-test were used to compare the control with the independent test item group.

**II. Results and Discussion**

**C. BIOLOGICAL EFFECTS**

In the control group and in the test item treatment group a parental mortality of 5.0 % and 1.3 %, respectively, could be observed at the end of the 14-day exposure period. Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 305.5 in the control and 311.5 in the test item treatment group. The test item caused no statistically significantly adverse effects on adult mortality (Fisher´s Exact Binomial test, α = 0.05, one-sided greater) and reproduction (Student-t-test, α = 0.05, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 1000 mg test item/kg soil dry weight. The results are summarized in Table A 90.

Table A 90: Effects of Propamocarb-hydrochloride SL 722 G on mortality and reproduction of predatory mites *(Hypoaspis aculeifer)* in a 14-day study

|  |  |  |
| --- | --- | --- |
| **Propamocarb-hydrochloride SL 722 G [mg/kg dry soil]** | **Control** | **1000** |
| Mean mortality (day 28) [%] | 5.0 | 1.3 |
| Mean no. of juveniles (day 28) | 305.5 | 311.5 |
| Reproduction in [%] of control (day 28) | 100 | 102 |
| Coefficient of variation of the number of juveniles [%] | 8.9 | 5.3 |
| **Endpoints** | **[mg test item/kg dry soil]** | |
| NOECmortality, reproduction | ≥ 1000 | |
| EC10 1) | > 1000 | |
| EC20 1) | > 1000 | |
| EC50 1) | > 1000 | |
| LC50 1) | > 1000 | |

1. Based on estimation.

No statistically significant differences compared to control were calculated (Fisher´s Exact Binomial Test for mortality, α = 0.05; Student-t-test for reproduction; α = 0.05)

Calculations were done using non-rounded values

In a separate study (BioChem project No. R 14 10 48 001 S, dated June 10, 2014), the EC50 (reproduction) of the reference item Dimethoate was calculated to be 6.2 mg/kg soil d.w. (tested concentrations: 1.00, 1.60, 2.56, 4.10, 6.55 and 10.5 mg/kg soil dry weight). The results of the reference test demonstrate the sensitivity of the test system.

**D. VALIDITY CRITERIA**

All validity criteria were met (Table A 91).

Table A 91: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 226 (2016)** | **Obtained in this study** |
| In the control(s), mean mortality of adult females must be ≤20% | 5.0% |
| In the control(s), mean number of juveniles per replicate must be ≥50 | 305.5 |
| In the control(s), the coefficient of variation for the mean number of juveniles per replicate must be ≤30% | 8.9% |

**E. DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

The effects of Propamocarb-hydrochloride SL 722 G on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a laboratory study over 14 days. The test item Propamocarb-hydrochloride SL 722 G showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 1000 mg test item/kg soil dry weight. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil dry weight.

* + - 1. KCP 10.4.2.2 Higher tier testing

Further studies were not triggered.

* 1. KCP 10.5 Effects on soil nitrogen transformation
     1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 216 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.5/01 |
| Report | Effects of BAS 743 02 F on the activity of soil microflora (Nitrogen transformation test  Schulz L., 2022  XXXX Study ID: 933752\_8  XXXX Doc ID: 2022/2033717 |
| Guideline(s): | OECD 216 (2000) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The effects of BAS 743 02 F on nitrogen transformation of soil microflora were determined over 28 days in the laboratory at two concentrations of 2.52 and 25.20 mg/kg product/kg soil dry weight (dw), corresponding to application rates of 1.01 and 10.08 mg/kg propamocarb and 0.32 and 3.20 mg/kg Ametoctradin (B, respectively. An untreated control was run in parallel. Each treatment group comprised of three replicates. Dinoterb was used as a reference item, tested in a separate study. Nitrogen transformation was determined as NO3-nitrogen-production in soil enriched with lucerne meal. NO3-nitrogen-production was measured at days 0, 7, 14 and 28 after application.

Exposure of BAS 743 02 F in a field soil up to and including the test concentration of 25.20 mg product/kg soil dw caused no adverse effects (deviation from control < 25 %) on the soil nitrogen transformation at the end of the 28-day incubation period.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Description:** | Soluble concentrate (SC) |
| **Lot/Batch:** | FRE-002224 |
| **Density:** | 1.080 g/cm³ |
| 1. **Control:** | Untreated substrate |
| 1. **Reference item:** | Dinoterb, 6.80, 13.60 and 27.20 mg/kg soil dw, tested in a separate study |

1. **study design and methods**

|  |  |
| --- | --- |
| 1. **Substrate** |  |
| **Soil type:** | Biologically active agricultural soil: sandy loam, 10.6% clay, 36.5% silt and 52.9% sand |
| **pH:** | 6.2 |
| **WHCmax (g/100 g soil dw):** | 37.57 |
| **Microbial biomass:** | 34.69 mg C/100 g soil dw  2.44% of total organic carbon |
| **Total organic carbon:** | 1.42% |
| **Total N (mg/kg dw):**  **Nmin (mg/100 g soil dw):** | 0.14  1.25 |
| **NH4+-N:** | Not reported |
| **NO2--N:** | Not reported |
| **NO3--N:** | 1.75 mg/100 g soil dw |
| 1. **Test units:** | The incubation of the prepared soil was carried out in wide-mouth glass flasks (500 mL). The screw caps of the flasks used permit an air exchange |
| 1. **Environmental conditions** |  |
| **Temperature:** | 19.5 - 20.8 °C |
| **Soil water content:** | 16.41 - 16.90 g/100 g soil dw (equivalent to 43.67 - 44.97 % of WHC) |
| **Photoperiod:** | 24 h darkness |

1. **Soil preparation and treatment:**

The soil was sampled at a depth of 20 cm from fallow land. Plant protection products had not been used on the land for approx. 33 years. The soil was dried at room temperature and passed through a 2 mm mesh sieve. It was then stored at approx. 4°C under anaerobic conditions in the dark until use. The soil was adapted to the test conditions prior to application of the test item.

The study comprised three treatment groups; two test item concentrations and an untreated control. The test item concentrations were 2.52 and 25.20 mg/kg product/kg soil dw, corresponding to application rates of 1.75 and 17.5 L product/ha, respectively. The active substance content in each treatment group are summarised in Table A 92.

Table A 92: Active substance content of the test item at each nominal test concentration

|  |  |  |
| --- | --- | --- |
| **BAS 743 02 F**  **(mg/kg soil dw)** | **Propamocarb**  **(mg/kg soil dw)** | **Ametoctradin /BAS 650 F**  **(mg/kg soil dw)** |
| 2.52 | 1.01 | 0.32 |
| 25.20 | 10.08 | 3.20 |

1. **Dose preparation:**

The incubation of the soil samples was performed as a series of individual and equally sized subsamples of each treatment group. 200 g soil dw (one sub-sample) per test vessel was weighed. The soil was mixed with 0.5% (i.e. 1.0 g/200 g soil dw) lucerne meal using a hand mixer. The C/N ratio of the lucerne meal was 13.2/1.

One additional soil sample (without lucerne meal) was used for determination of the initial NH4 and NO3 content. The test item was mixed with deionised water and the test solution was subsequently mixed with the soil using a hand mixer. Water was added to the soil to achieve a water content of approximately 45% of WHC.

1. **Measurements and observations:**

Soil samples (10 g soil dw per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH4-N, NO3-N and NO2-Ncontents were determined (depending on treatment group). Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g soil dw and mixing on a rotator at 150 rpm for 60 minutes. The mixtures were centrifuged, and the supernatant was stored deep-frozen prior to analysis at -20 ± 5°C. For the quantitative determination of the mineralized part of nitrogen, an autoanalyser was used. The autoanalyser was calibrated before each measurement series. The LOQ for nitrogen and ammonium were 1.03 mg/100 g soil dw and 0.16 mg/100 g soil dw, respectively.

The water content of the soil in each test vessel was determined at test start (after application) and adjusted once per week to the required range of 40 - 50% of WHC. The pH of the soil was measured at test start (after application) and on day 28.

1. **Statistical analysis:**

Descriptive statistics were used. The mean nitrogen content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date.

**II. Results and Discussion**

1. **biological effects**

No adverse effects of BAS 743 02 F on nitrogen transformation in soil could be observed at both test concentrations (2.52 mg/kg dry soil and 25.20 mg/kg dry soil) after 28 days (time interval 0-28). Only negligible deviations from the control of +5.6 % (test concentration 2.52 mg/kg dry soil) and +7.6 % (test concentration 25.20 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 0-28).

Table A 93: Effects on nitrogen transformation in soil after treatment with BAS 743 02 F

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time interval  (days)** | **Control** | **BAS 743 02 F 2.52 mg/kg soil dw** | | **BAS 743 02 F 25.20 mg/kg soil dw** | |
| **NO3 in mg/kg soil dw 1)** | **NO3 in mg/kg soil dw** **1)** | **% deviation from control** | **NO3 in mg/kg soil dw 1)** | **% deviation from control** |
| **0-7** | 2.96 | 3.34 | +12.7 | 3.54 | +19.5 |
| **0-14** | 2.31 | 2.65 | +14.8 | 2.75 | +19.2 |
| **0-28** | 1.96 | 2.07 | +5.6 | 2.10 | +7.6 |

1) (measured values sampling day “x” - measured values sampling day 0) / days, mean of 3 replicates

The calculations were performed with unrounded values

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of +37.3 % and +29.5 % at 13.60 mg/kg and 27.20 mg/kg soil dry weight determined 28 days after application. Thus, the results of the reference test demonstrated the sensitivity of the test system.

1. **VALIDITY CRITERIA**

The validity criteria were met (Table A 94)

Table A 94 Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 216 (2000)** | **Obtained in this study** |
| In the control(s), the coefficients of variation for NO3 must be <15% | 6.9% |

1. **DEFICIENCIES**

There were no deficiencies.

**III. Conclusion**

The effects of BAS 743 02 F on nitrogen transformation of soil microorganisms were determined over 28 days at two test item concentrations of 2.52 and 25.20 mg/kg product/kg soil dw, corresponding to application rates of 1.75 and 17.5 L product/ha, respectively. The test item caused no adverse effects (deviation from control < 25 %) on soil nitrogen transformation of soil microorganisms (measured as NO3 production) in the field soil up to a test concentration of 25.20 mg/kg soil dw at the end of the 28-day incubation period.

* 1. KCP 10.6 Effects on terrestrial non-target higher plants
     1. KCP 10.6.1 Summary of screening data
        1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 208 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.6.1/01 |
| Report | Effect of BAS 743 02 F on seedling emergence and seedling growth of several species of terrestrial plants under greenhouse conditions  Maleck, A., 2023a  XXXX Study ID: 933752-13  XXXX Doc ID: 2022/2033722 |
| Guideline(s): | OECD 208 (2006); OCSPP 850.4100 (2012) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The effects of pre-emergence exposure of BAS 743 02 F on seedling emergence and growth of six dicotyledonous and four monocotyledonous plant species were tested in a limit test design. BAS 743 02 F was applied pre-emergence shortly after sowing at 3.85 L product/ha. A control (tap water only) was tested in parallel. Plants were cultivated for 21 days under greenhouse conditions. Based on the results of this study, it can be concluded that the fungicide BAS 743 02 F applied at a rate of 3.85 L product/ha did not cause effects on seedling emergence, plant survival, visual phytotoxicity, plant development (BBCH), plant length and plant dry biomass of the tested plant species.

For all tested species an ER50 > 3.85 L/ha based on the aforementioned endpoints was determined.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Description:** | Liquid |
| **Lot/Batch:** | FRE-002224 |
| **Density:** | 1.080 g/cm³ |
| 1. **Control:** | Tap water |

**B. STUDY DESIGN AND METHODS**

|  |  |
| --- | --- |
| 1. **Test soil:** |  |
| **Soil type:** | Natural soil (poor silty sand) |
| **Particle size distribution:** | 4.9% <2 µm, 20.2% 2-63 µm, 75.0% >63 µm |
| **Total organic carbon:** | 0.8% |
| **pH:** | 7.18 |
| 1. **Test units:** | Plastic containers (5 cm diameter) |
| 1. **Environmental conditions** |  |
| **Temperature:** | Daily mean 22.9°C to 28.8°C |
| **Relative humidity:** | Daily mean 57.0 % to 78.7 % |
| **Photoperiod:** | The natural day length during the study period was adjusted to ≥16 hours by adding artificial light for 16 hours in maximum if indoor light intensity was lower than 300 µmol/m2s. |

1. **Soil preparation and treatment:**

The test was set up with six dicotyledonous and four monocotyledonous species, representing eight different plant families. There were four replicates per treatment, each one consisting in one, two or three pots with ten (carrot, onion, ryegrass, wheat), five (lettuce, oilseed rape, soybean, tomato, corn) or four (cucumber) seeds, respecitvley. Plant species were sown immediately before application. Each pot was placed on a separate tray and irrigated only from the bottom. After application all pots per plant species were set to a greenhouse table in a randomized design.

BAS 743 02 F was applied to all plant species on pre-emergence and shortly after sowing using a laboratory application chamber at a spray volume of 286 L/ha. The tested rate was 3.85 L product/ha. Per plant species one control (tap water only) was tested.

Table A 95: Overview of test species

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Family** | **Species** | **Common name** |
| Dicotyledonae | Apiaceae | *Daucus carota* | Carrot |
| Dicotyledonae | Asteraceae | *Lactuca sativa* | Lettuce |
| Dicotyledonae | Brassicaceae | *Brassica napus* ssp. *napus* | Oilseed rape |
| Dicotyledonae | Cucurbitaceae | *Cucumis sativus* | Cucumber |
| Dicotyledonae | Fabaceae | *Glycine max* | Soybean |
| Dicotyledonae | Solanaceae | *Solanum lycopersicum*. | Tomato |
| Monocotyledonae | Amaryllidaceae | *Allium cepa* | Onion |
| Monocotyledonae | Poaceae | *Lolium multiflorum* | Rye grass |
| Monocotyledonae | Poaceae | *Triticum aestivum* | Wheat |
| Monocotyledonae | Poaceae | *Zea mays* | Corn |

1. **Dose preparation:**

Based on the mean output volume of the sprayer (2786 L/ha) the required amount of the test item was calculated based on the mix volume used for application. For this mix volume the amount of test item was weighed and dissolved in the respective water volume.

1. **Measurements and observations:**

Biological observations:

General first assessment per species was done when at least 50 % of the planted seeds of the untreated control reached the BBCH stage 10 (equals to 0 days after emergence). All following assessments were performed at 7, 14 and 21 days after emergence (DAE) except assessments of plant length and biomass at

21 DAE.

Assessments of phytotoxicity were done weekly after emergence following a scale of 0 – 100 % where 0% means no difference to untreated and 100% plant is completely destroyed. Damage was categorized as chlorosis (any kind of discoloration), necrosis, deformation and others (no growth reduction).

Plant length of all living plants was measured in cm as single plant length. For assessment for biomass, all living plants per replicate were cut directly at the soil surface and dry weight was determined immediately after mass constancy (at 60 °C) of the plants was reached. All plants of each replicate were weighed together.

Physicochemical measurements:

In the greenhouse chamber temperature and humidity had been documented using an electronic data logger device.

Analytical verification:

Samples of spray solution were taken from the untreated control and the test item treatment before application and were stored at < -18°C until they were transferred to the analytical laboratory. The analytical verification was done by analysing the concentration of the active substance ametoctradin (BAS 650) by LC-MS/MS.

1. **Statistics:**

Descriptive statistics were performed using Microsoft EXCEL software.

Plant survival was calculated within statistical procedures as compared to total emerged plants per replicate. If the application of the test item had a possible negative effect on seedling emergence, plant survival, plant length or biomass compared to untreated control, further statistical analysis was carried out using ToxRat Standard (version 3.3.0) of ToxRat Solutions GmbH, Germany.

Pretesting sequences for metric data were a test on normal distribution (Shapiro-Wilk’s test, p=0.01) and a variance homogeneity check (Levene’s test, p=0.01). Depending on outcomes, the limit concentration of BAS 743 02 F for emergence, plant survival, plant length and biomass was tested by pairwise comparison with the control. Metric data were tested by Two-sample t-test (Student t-test, one-sided smaller, p = 0.01). Quantal data were tested by Two-sample Fisher’s Exact test (one-sided greater, p = 0.01).

1. **Description of the analytical procedures**

The analysis of BAS 743 02 F was performed via its active substance BAS 650 F (ametoctradin) and was based on XXXX method No. L0208/02 for determination of residues of the analyte in water. The method was adapted to perform the analysis in the given concentration range. The aqueous application solutions were diluted in three steps by a total factor of 100000 using 30 μL of silicon antifoam emulsion in 1.0 L of tap water as well as acetonitrile/water (50/50, v/v) as solvent. Five replicate dilutions were analyzed for each selected application solution. The diluted application solutions were analysed for the content of BAS 650 F by LC-MS/MS with external standardisation. Linear calibration curve(s) with a correlation coefficient r > 0.99 were used for quantification. Due to the high total dilution factor (100000), no relevant matrix effects were observed for the LC-MS/MS determination of BAS 650 F. The average concentration of the active substance BAS 650 F (ametoctradin) obtained by LC-MS/MS analysis of the application solution derived from the test item BAS 743 02 F was 1.63 g/L (nominal: 1.85 g/L BAS 650 F) equivalent to a recovery of 87.9 % of the expected concentration. No analyte (above the defined LOD of 0.05 g/L) or relevant interferences were detected in the LC-MS/MS analysis of a respective blank control solution.

Table A 96: Procedural recoveries for BAS 743 02 F in Fortified Recovery Samples by LC-MS/MS

| **Fortification level (g/L)** | **n** | **Average (%)** | **±SD** | **RSD (%)** |
| --- | --- | --- | --- | --- |
| 0.180 (LOQ) | 5 | 95.1 | 4.0 | 4.88 |
| 3.56 | 5 | 103 | 1.2 | 1.62 |

RSD = Relative standard deviation

1. **Results and Discussion**
2. **Biological effects**

None of the tested plant species was affected concerning seedling emergence and plant survival by the pre-emergence application of 3.85 L product/ha. No phytotoxicity, reduction of plant length or dry weight was observed for all tested plant species following the application of 3.85 L product/ha. The NOER of plant emergence, plant survival, plant length, plant biomass reduction and phytotoxicity for all tested plant species is higher than the tested rate of 3.85 L BAS 743 02 F/ha. The biological effects are summarised in Table A 97 below.

Table A 97: Summary of plant emergence, survival, length and dry weight following exposure to BAS 743 02 F

| **Plant species** | **Rate of BAS 743 02 F**  **[L product/ha]** | **Emerged plants per replicate**  **0-21 DAE** | **Emergence compared to control [%]** | **Survival**  **[%]** | **Phytotoxic effects**  **[%]** | **Length [cm]** | **Biomass (dry)**  **[g]** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Carrot | 0.0 | 8.3 | - | 100 | 0 | 21.2 | 3.941 |
| 3.85 | 8.3 | 83 | 100 | 0 | 21.2 | 3.923 |
| Lettuce | 0.0 | 10.0 | - | 100 | 0 | 12.9 | 6.916 |
| 3.85 | 10.0 | 100 | 100 | 0 | 12.7 | 6.858 |
| Oilseed rape | 0.0 | 8.0 | **-** | 100 | 0 | 26.7 | 40.466 |
| 3.85 | 8.5 | 106 | 100 | 0 | 26.1 | 39.995 |
| Cucumber | 0.0 | 10.5 | - | 100 | 0 | 18.4 | 25.067 |
| 3.85 | 10.0 | 95 | 100 | 0 | 17.6 | 22.991 |
| Soybean | 0.0 | 8.5 | - | 100 | 0 | 35.2 | 22.881 |
| 3.85 | 8.5 | 100 | 100 | 0 | 35.4 | 22.911 |
| Tomato | 0.0 | 9.3 | - | 100 | 0 | 28.5 | 25.398 |
| 3.85 | 9.5 | 103 | 100 | 0 | 28.3 | 26.326 |
| Onion | 0.0 | 8.5 | - | 100 | 0 | 20.3 | 0.753 |
| 3.85 | 8.3 | 97 | 100 | 0 | 21.5 | 0.852 |
| Ryegrass | 0.0 | 9.3 | - | 100 | 0 | 43.6 | 6.765 |
| 3.85 | 9.3 | 100 | 100 | 0 | 43.0 | 6.076 |
| Wheat | 0.0 | 9.8 | - | 100 | 0 | 36.4 | 8.652 |
| 3.85 | 10.0 | 103 | 100 | 0 | 36.2 | 8.671 |
| Corn | 0.0 | 10.0 | - | 100 | 0 | 108.2 | 89.474 |
| 3.85 | 10.0 | 100 | 100 | 0 | 109.5 | 87.676 |

Treatment not significantly different to control

1. **ANALYTICAL RESULTS**

The laboratorial verification of the spray concentration at the tested rate was 87.9 % and thus reflecting the theoretical value. In the untreated specimen (control), no residue of BAS 743 02 F was found.

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 98: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 208 (2006)** | |
| Germination Rate of the Control Seeds: | ≥ 70%; validity criterion was met (observed: 80-100%). |
| Mean Survival of Emerged Control Seedlings: | ≥ 90%, validity criterion was met (observed: 100 %). |
| Growth and Morphology of the Control Plants: | The control seedlings exhibited no visible phytotoxic effects |

1. **DEFICIENCIES**

None.

**III. CONCLUSION**

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that the fungicide BAS 743 02 F applied pre-emergence at a rate of 3.85 L product/ha did not cause effects to the seedling emergence, plant survival, visual phytotoxicity, plant development (BBCH), plant length and plant dry biomass of the tested plant species. For all tested species, an ER50 > 3.85 L product/ha based on the aforementioned endpoints was determined.

* + - 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD 227 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Data point: | CP 10.6.1/02 |
| Report | Effect of BAS 743 02 F on vegetative vigour of several species of terrestrial plants under greenhouse conditions  Maleck, A., 2023b  XXXX Study ID: 933752-14  XXXX Doc ID: 2022/2033723 |
| Guideline(s): | OECD 227 (2006); OCSPP 850.4150 (2012) |
| Deviations: | No |
| GLP: | Yes, GLP |
| Acceptability: | Yes |

**Executive Summary**

The effect of BAS 743 02 F on vegetative vigour of ten species of terrestrial plants was assessed in a limit test study. BAS 743 02 F was applied post-emergence at BBCH 12 -14 at 3.85 L product/ha. A control (tap water only) was tested in parallel. Plants were cultivated for 21 days under greenhouse conditions. Assessments for plant damage (phytotoxicity), plant development and plant survival were done 7, 14 and 21 days after treatment (DAT). Single plant length and dry weight were measured at 21 DAT. Based on the results of this study, it can be concluded that BAS 743 02 F applied post emergence at a rate of 3.85 L product/ha did not cause effects to plant phytotoxicity, plant survival, plant length and plant dry biomass for all tested plant species.

1. **MATERIALS AND METHODS**
2. **MATERIALS**

|  |  |
| --- | --- |
| 1. **Test item:** | BAS 743 02 F |
| **Active substance content:** | Propamocarb: nominal 432.0 g/L, analysed 431.0 g/L;  Ametoctradin: nominal 137.14 g/L, analysed 137.7 g/L |
| **Description:** | Liquid |
| **Lot/Batch:** | FRE-002224 |
| **Density:** | 1.080 g/cm³ |
| 1. **Control:** | Tap water |

**B. STUDY DESIGN AND METHODS**

|  |  |
| --- | --- |
| 1. **Test soil:** |  |
| **Soil type:** | Natural soil (poor silty sand) |
| **Particle size distribution:** | 45.5% <2 µm, 19.4% 2-63 µm, 75.1% >63 µm |
| **Total organic carbon:** | 1.7% |
| **pH:** | 7.4 |
| 1. **Test units:** | Plastic containers (5 cm diameter) |
| 1. **Environmental conditions** |  |
| **Temperature:** | Daily mean 21.0 °C - 28.3 °C |
| **Relative humidity:** | Daily mean 56.8 % - 87.3 % |
| **Photoperiod:** | Photoperiod: day length > 16 hours; additional light supply automatically for 16 hours in maximum when indoor illumination was less than 300 µmol |

1. **Soil preparation and treatment:**

The test was set up with six dicotyledonous and four monocotyledonous species, representing eight different plant families. There were five replicates per treatment, each one consisting in one, three or two pots with six (carrot, onion, ryegrass, wheat), two (cucumber, soybean, tomato, corn) or three (lettuce, oilseed rape) plants, respectively. Test crops were seeded several days before application to gain plants at BBCH stage 12 - 14. Each pot was placed on a separate tray and irrigated only from the bottom. After application all pots per plant species were set to a greenhouse chamber in a randomized design.

BAS 743 02 F was applied to all plant species at BBCH stage 12-14 using a laboratory application chamber at a spray volume of 286 L/ha. The tested rate was 3.85 L product/ha. Per plant species one control (tap water only) was tested.

Table A 99: Overview of test species

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Family** | **Species** | **Common name** |
| Dicotyledonae | Apiaceae | *Daucus carota* | Carrot |
| Dicotyledonae | Asteraceae | *Lactuca sativa* | Lettuce |
| Dicotyledonae | Brassicaceae | *Brassica napus* ssp. *napus* | Oilseed rape |
| Dicotyledonae | Cucurbitaceae | *Cucumis sativus* | Cucumber |
| Dicotyledonae | Fabaceae | *Glycine max* | Soybean |
| Dicotyledonae | Solanaceae | *Solanum lycopersicum*. | Tomato |
| Monocotyledonae | Amaryllidaceae | *Allium cepa* | Onion |
| Monocotyledonae | Poaceae | *Lolium multiflorum* | Rye grass |
| Monocotyledonae | Poaceae | *Triticum aestivum* | Wheat |
| Monocotyledonae | Poaceae | *Zea mays* | Corn |

1. **Dose preparation:**

Based on the mean output volume of the sprayer (286L/ha) the required amount of the test item was calculated based on the mix volume used for application. For this mix volume the amount of test item was weighed using and was dissolved in the respective water volume.

1. **Measurements and observations:**

Biological observations:

All assessments were performed at 7, 14 and 21 days after treatment (DAT) except assessments of plant length and biomass at 21 DAT.

Plant survival was determined per replicate. To be considered alive, a plant had to have at least one green part (not complete necrotic). Plant development (BBCH) was determined as range per replicate. Assessments for visual phytotoxicity were done following a scale of 0 – 100% (with 0% = no effects and 100% = all plants dead). Visual phytotoxicity was categorised as chlorosis (any kind of discoloration), necrosis, deformation and others (no growth reduction). Plant length of all living plants was measured in cm as single plant length. For assessment for biomass, all living plants per replicate were cut directly at the soil surface and dry weight was determined immediately after mass constancy (at 60 °C) of the plants was reached. All plants of each replicate were weighed together.

Physicochemical measurements:

In the greenhouse chamber temperature and humidity had been documented using an electronic data logger device.

Analytical verification:

Samples of spray solution had been taken from the untreated control and the test item treatment before application and were stored at < -18°C until they were transferred to the analytical laboratory. The analytical verification was done by analysing the concentration of the active substance ametoctradin (BAS 650) by LC-MS/MS.

1. **Statistics:**

Descriptive statistics regarding number of plants, plant length and biomass were performed using Microsoft EXCEL.

If the application of the test item had a possible negative effect on plant survival, plant length or biomass compared to untreated control, further statistical analyses were carried out using ToxRat Standard (version 3.3.0) of ToxRat Solutions GmbH, Germany.

Pretesting sequences for metric data were a test on normal distribution (Shapiro-Wilk’s test, p=0.01) and a variance homogeneity check (Levene’s test, p=0.01). Depending on outcomes of pretesting sequences the limit concentration of BAS 743 02 F for plant length and biomass was tested by pairwise comparison with the control. Metric data were tested by Two-sample t-test (Student t-test, one-sided smaller, p = 0.01). No statistical evaluation was necessary for quantal data due to the lack of mortality.

1. **Description of the analytical procedures**

The analysis of BAS 743 02 F was performed via its active substance BAS 650 F (ametoctradin) was based on XXXX method No. L0208/02 for determination of residues of the analyte in water. The method was adapted to perform the analysis in the given concentration range. The aqueous application solutions were diluted in three steps by a total factor of 100000 using 30 μL of silicon antifoam emulsion in 1.0 L of tap water as well as acetonitrile/water (50/50, v/v) as solvent. Five replicate dilutions were analysed for each selected application solution. The diluted application solutions were analysed for the content of BAS 650 F by LC-MS/MS with external standardisation. Linear calibration curve(s) with a correlation coefficient r > 0.99 were used for quantification. Due to the high total dilution factor (100000), no relevant matrix effects were observed for the LC-MS/MS determination of BAS 650 F. The average concentration of the active substance BAS 650 F (ametoctradin) obtained by LC-MS/MS analysis of the application solution derived from the test item BAS 74 3 02 F was 1.54 g/L (nominal: 1.85 g/L BAS 650 F) equivalent to a recovery of 83.2 % of the expected concentration. No analyte (above the defined LOD of 0.05 g/L) or relevant interferences were detected in the LC-MS/MS analysis of a respective blank control solution.

Table A 100: Procedural recoveries for BAS 743 02 F in Fortified Recovery Samples by LC-MS/MS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Fortification level (g/L)** | **n** | **Average (%)** | **±SD** | **RSD (%)** |
| 0.179 (LOQ) | 5 | 103 | 4.36 | 4.79 |
| 3.79 | 5 | 91.5 | 2.59 | 3.16 |

RSD = Relative standard deviation

1. **Results AND Discussion**
2. **Biological effects**

No negative impact of BAS 743 02 F on plant survival, plant length and dry biomass production was observed for all tested species after the application of 3.85 L BAS 743 02 F/ha at BBCH stage 12-14. The biological effects are summarised in Table A 101below.

Table A 101 Summary of plant survival, length and dry weight following exposure to BAS 743 02 F at BBCH stage 12-14

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Plant species** | **Rate of BAS 743 02 F**  **[L product/ha]** | **Survival**  **[%]** | **Phytotoxic effects**  **[%]** | **Length**  **[cm]** | **Biomass (dry)**  **[g]** |
| Carrot | 0.0 | 100 | 0 | 45.2 | 17.220 |
| 3.85 | 100 | 0 | 43.9 | 17.426 |
| Lettuce | 0.0 | 100 | 0 | 22.0 | 36.424 |
| 3.85 | 100 | 0 | 22.2 | 35.253 |
| Oilseed rape | 0.0 | 100 | 0 | 42.3 | 110.716 |
| 3.85 | 100 | 0 | 42.6 | 108.641 |
| Cucumber | 0.0 | 100 | 0 | 174.3 | 160.079 |
| 3.85 | 100 | 0 | 172.7 | 144.487 |
| Soybean | 0.0 | 100 | 0 | 86.9 | 71.339 |
| 3.85 | 100 | 0 | 77.9 | 72.516 |
| Tomato | 0.0 | 100 | 0 | 75.2 | 112.105 |
| 3.85 | 100 | 0 | 74.6 | 113.965 |
| Onion | 0.0 | 100 | 0 | 38.8 | 10.821 |
| 3.85 | 100 | 0 | 39.4 | 11.508 |
| Ryegrass | 0.0 | 100 | 0 | 57.1 | 18.941 |
| 3.85 | 100 | 0 | 58.2 | 18.192 |
| Wheat | 0.0 | 100 | 0 | 52.8 | 18.115 |
| 3.85 | 100 | 0 | 52.6 | 18.952 |
| Corn | 0.0 | 100 | 0 | 162.0 | 145.550 |
| 3.85 | 100 | 0 | 159.0 | 137.565 |

Treatment not significantly different to control

1. **ANALYTICAL RESULTS**

The laboratorial verification of the spray concentration at the tested rate was 83 % and thus reflecting the theoretical value. In the untreated specimen (control), no residue of BAS 743 02 F was found.

1. **VALIDITY CRITERIA**

All validity criteria were met.

Table A 102: Validity criteria

|  |  |
| --- | --- |
| **Validity criteria according to OECD 227 (2006)** | |
| Plant survival in the untreated control: | ≥ 90%; validity criterion was met (observed: 100%). |
| Seedling emergence rate: | ≥ 70%, validity criterion was met (observed: 80-100 %). |
| Growth and Morphology of the Control Plants: | The control seedlings exhibited no visible phytotoxic effects |

1. **DEFICIENCIES**

None.

1. **CONCLUSION**

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that BAS 743 02 F applied post-emergence at a rate of 3.85 L product/ha did not cause effects to visual plant phytotoxicity, plant survival, plant length and plant dry biomass for all tested plant species. For all tested species an ER50 > 3.85 L product/ha based on the aforementioned endpoints was determined.

* + 1. KCP 10.6.2 Testing on non-target plants

No new studies are available.

* + 1. KCP 10.6.3 Extended laboratory studies on non-target plants

As BAS 743 03 F does not pose an unacceptable risk to non-target plants, further studies are not necessary.

* + 1. KCP 10.6.4 Semi-field and field tests on non-target plants

As BAS 743 03 F does not pose an unacceptable risk to non-target plants, further studies are not necessary.

* 1. KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No further data submitted.

* 1. KCP 10.8 Monitoring data

According to the knowledge of the applicant, there are currently no monitoring studies available which assess ecotoxicological effects of BAS 743 03 F or of the active substances.

1. J.M. Buxton, D.R. Crocker & J. A. Pascual Birds and farming: information for risk assessment, 1998 Update CONTRACT PN0919 MILESTONE REPORT, CSL Project No. M37 [↑](#footnote-ref-1)
2. European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu. [↑](#footnote-ref-2)
3. Leutert A. (1983). Einfluss der Feldmaus, Microtus arvalis (Pall.), auf die floristische Zusammensetzung von Wiesen-Ekosystemen. Veröffentlichung des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel, Zürich [↑](#footnote-ref-3)
4. Rinke, T. (1991): Percentage of volume versus number of species: Availability and intake of grasses and forbs in *Microtus arvalis*. Folia zoologica 40 (2): 143-151. [↑](#footnote-ref-4)
5. Rinke T. (1990). Zur Nahrungsökologie von *Microtus arvalis* (Pallas, 1779) auf Dauergrünland. *Z. Säugetierkunde*, 55:106-116 [↑](#footnote-ref-5)
6. European Food Safety Authority, 2013 (update 2014). 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, 268 pp., doi:10.2903/j.efsa.2013.3295. [↑](#footnote-ref-6)
7. EFSA (2020): Bee-Tool v.3 available at http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3295/full [↑](#footnote-ref-7)
8. Kyriakopoulou, K., Kandris, I., Pachiti, I., Kasiotis, K.M., Spyropoulou, A., Santourian, A., Kitromilidou, S., Pappa, G. and Glossioti, M. "Collection and analysis of pesticide residue data for pollen and nectar–Final Report." EFSA Supporting Publications 14.10 (2017). [↑](#footnote-ref-8)