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| REGISTRATION REPORT  Part B  Section 9  Ecotoxicology  Detailed summary of the risk assessment |
| Product code: BAS 736 00 F  Product name(s): **Miralon**  Chemical active substance(s):  Fluxapyroxad, 50 g/L Azoxystrobin, 75 g/L |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT  (new authorization) |
| Applicant: BASF  Submission date: 08/2022  Evaluation date: August 2022  MS Finalisation date: January 2023 |

Version history

|  |  |
| --- | --- |
| When | What |
| 12/2021 | Initial dRR - BASF DocID 2021/2048070 |
| 08/2022 | Updated version - BASF DocID 2022/2039019 |
| 09/2022 | Version evaluated by zRMS PL |
| 01/2023 | Version updated after commenting proces. |

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# Ecotoxicology (KCP 10)

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| **Review Comments:**  This document describes the acceptable use conditions required for registration of BAS 736 00 F, an emulsifiable concentrate containing 50 g/L fluxapyroxad (BAS 700 F) and 75 g/L azoxystrobin (BAS 9164 F) for use as a fungicide in cereals.  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 9.1‑1: Table of critical GAPs

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 18 | 20 | 21 |
| **Use-No. (e)** | **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  (f) | **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 | DE, AT, BE, NL, IE, PL | wheat TRZAW, TRZAS TRZDU, TRZSP | F | Zymoseptoria tritici - SEPTTR Puccinia triticina - PUCCRT Puccinia striiformis - PUCCST Pyrenophora tritici-repentis – PYRNTR  Blumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 2.00 b) 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 2 | DE, AT, BE, NL, IE | barley  HORVW  HORVS | F | Pyrenophora teres – PYRNTE  R. secalis - RHYNSER. collo-cygni – RAMUCC  Puccinia hordei – PUCCHD  Blumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 2  b) 2 | 21 | a) 2.00  b) 4.00 | a) 0.100 / 0.150b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 3 | DE, AT, BE, NL, IE, PL | rye SECCW SECCS SECCE | F | R. secalis - RHYNSE Puccinia recondita - PUCCRE | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 2.00 b) 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 4 | DE, AT, BE, NL, IE, PL | triticale TTLWI  TTLSO | F | Septoria spp. - SEPTSP Puccinia recondita - PUCCRE Puccinia striiformis – PUCCST  Blumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 2.00 b) 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 5 | DE, AT, BE, NL, IE | oat AVESA | F | Blumeria graminis - ERYSGR  Puccinia coronata - PUCCCA | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 2.00 b) 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 6 | PL | barley HORVW  HORVS | F | Pyrenophora teres – PYRNTE  R. secalis – RHYNSE  Puccinia hordei - PUCCHD R. collo-cygni - RAMUCC | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 2.00 b) 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 7 | CZ | wheat TRZAW, TRZAS TRZDU, TRZSP | F | Zymoseptoria tritici - SEPTTRPuccinia triticina - PUCCRTPuccinia striiformis - PUCCSTPyrenophora tritici-repentis – PYRNTR  Blumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 1 b) 1 |  | a) 1.20 - 2.00 b) 1.20 - 2.00 | a) 0.100 / 0.150 b) 0.100 / 0.150 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 8 | CZ | barley HORVW  HORVS | F | Pyrenophora teres – PYRNTER. secalis - RHYNSER. collo-cygni – RAMUCC  Puccinia hordei – PUCCHDBlumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 1 b) 1 |  | a) 1.20 - 2.00 b) 1.20 - 2.00 | a) 0.100 / 0.150 b) 0.100 / 0.150 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 9 | CZ | rye SECCW SECCS SECCE | F | R. secalis - RHYNSEPuccinia recondita - PUCCRE | Spraying (SP) | 30 - 69 | a) 1 b) 1 |  | a) 1.20 - 2.00 b) 1.20 - 2.00 | a) 0.100 / 0.150 b) 0.100 / 0.150 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 10 | CZ | triticale TTLWI  TTLSO | F | Septoria spp. - SEPTSP Puccinia recondita - PUCCRE Puccinia striiformis – PUCCST  Blumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 1 b) 1 |  | a) 1.20 - 2.00 b) 1.20 - 2.00 | a) 0.100 / 0.150 b) 0.100 / 0.150 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 11 | CZ | oat AVESA | F | Blumeria graminis - ERYSGR  Puccinia coronata - PUCCCA | Spraying (SP) | 30 - 69 | a) 1 b) 1 |  | a) 1.20 - 2.00 b) 1.20 - 2.00 | a) 0.100 / 0.150 b) 0.100 / 0.150 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 12 | HU, SI, SK, RO | wheat TRZAW, TRZAS TRZDU, TRZSP | F | Zymoseptoria tritici - SEPTTR Puccinia triticina - PUCCRT Puccinia striiformis - PUCCST | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 1.00 - 2.00 b) 1.00 - 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 13 | HU, SI, SK, RO | barley HORVW  HORVS | F | Pyrenophora teres - PYRNTE Puccinia hordei – PUCCHD  Blumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 1.00 - 2.00 b) 1.00 - 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 14 | HU, SI, SK, RO | rye SECCW SECCS SECCE | F | R. secalis - RHYNSE Puccinia recondita - PUCCRE | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 1.00 - 2.00 b) 1.00 - 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 15 | HU, SI, SK, RO | triticale TTLWI  TTLSO | F | Zymoseptoria sp. – SEPTSP  Puccinia recondita – PUCCRE  Puccinia striiformis – PUCCST  Blumeria graminis - ERYSGR | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 1.00 - 2.00 b) 1.00 - 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |
| 16 | HU, SI, SK, RO | oat AVESA | F | Blumeria graminis - ERYSGR Puccinia coronata - PUCCCA | Spraying (SP) | 30 - 69 | a) 2 b) 2 | 21 | a) 1.00 - 2.00 b) 1.00 - 4.00 | a) 0.100 / 0.150 b) 0.200 / 0.300 | 100 - 300 | 35 |  |  |  |  |  |  |  |  |

\* 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

1) Fluxapyroxad

2) Azoxystrobin

# only for scenario R3

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*

Exposure to active substances separately

In the screening step all TERA values and all TERlt values for fluxapyroxad and azoxystrobin exceed the trigger values set by Commission Regulation (EU) 546/2011 for acceptability of effects.

Exposure to combined active substances and to the formulation

The two acute risk assessment approaches carried out (combined toxicity of the active substances as virtual compound and formulation toxicity) have resulted in TER values at the screening and/or tier 1 acute risk assessment above the trigger of 10 for acceptability of effects. The reproductive risk assessment using the concentration addition model resulted in TER value above the trigger of 5 for acceptability of effects in screening step.

Therefore, the acute and reproductive dietary risk to birds from BAS 736 00 F according to the proposed use pattern is acceptable.

*Drinking water risk assessment*

Following EFSA/2009/1438, the puddle scenario is considered relevant for applications of BAS 736 00 F according to the proposed use pattern. Since the ratio of the effective application rate to the relevant toxicity endpoints is below the values of 3000 and 50 for fluxapyroxad and azoxystrobin, respectively, a quantitative risk assessment for the proposed use pattern of BAS 736 00 F is not necessary.

*Secondary poisoning and biomagnification*

The log Pow of the active substance fluxapyroxad is > 3, 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 fluxapyroxad are above the trigger value of 5, indicating an acceptable risk for the intended use of BAS 736 00 F. The log Pow of the active substance azoxystrobin is < 3, thus, a risk assessment for effects due to secondary poisoning is not required. Low potential for accumulation of fluxapyroxad and azoxystrobin in animal tissue was concluded in the respective EU reviews and therefore further evaluation of biomagnification is not necessary.

***Overall conclusion***

**It can be concluded that the risk to birds from the application of BAS 736 00 F according to good agricultural practice is acceptable.**

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

*Dietary risk assessment*

Exposure to active substances separately

In the screening step and/or tier 1 risk assessment, all TERA values and all TERLT values for fluxapyroxad and azoxystrobin exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects.

Exposure to combined active substances and to formulation

For the acute risk assessment addressing combined toxicity of the active substances as virtual compound the TERA value for the screening step is above the trigger of 10.

For the acute risk assessment addressing combined toxicity of the active substances in the formulation the tier 1 risk assessment resulted in TERA values above the trigger of 10 for acceptability of effects for all scenarios, except for the small herbivorous mammal “vole” scenario at BBCH ≥ 40.

The two different refinement approaches are presented. In the first approach the acute higher tier risk assessment for the formulation is based on the refinement of the deposition factor, whereas the second approach is based on the interpolated LD50 value for BAS 736 00 F as refinement parameter. Both approaches resulted in refined TERA values for the formulation toxicity of BAS 736 00 F that are above the trigger of 10 for acceptability of effects for the small herbivorous mammal “vole” scenario at BBCH ≥ 40.

The reproductive risk assessment using the concentration addition model resulted in an acceptable risk for all scenarios, except for the small herbivorous mammal “vole” scenario at BBCH ≥ 40. Based on the higher tier risk assessment considering the refinement of the deposition factor an acceptable combined reproductive risk to small herbivorous mammal “vole” could be identified.

Therefore, the acute and reproductive dietary risk to mammals from BAS 736 00 F according to the proposed use pattern is acceptable.

*Drinking water risk assessment*

Following EFSA/2009/1438, the puddle scenario is the one relevant for mammals. Since the ratio of the effective application rate to the relevant toxicity endpoints is below the values of 3000 and 50 for fluxapyroxad and azoxystrobin, respectively, a quantitative risk assessment for the proposed use pattern of BAS 736 00 F is not necessary.

*Secondary poisoning and biomagnification*

The log Pow of the active substance fluxapyroxad is > 3, 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 fluxapyroxad are above the trigger value of 5, indicating an acceptable risk for the intended use of BAS 736 00 F. The log Pow of the active substance azoxystrobin is < 3, thus, a risk assessment for effects due to secondary poisoning is not required. Low potential for accumulation of fluxapyroxad and azoxystrobin in animal tissue was concluded in the respective EU reviews and therefore further evaluation of biomagnification is not necessary.

***Overall conclusion***

**It can be concluded that the risk to mammals from the application of BAS 736 00 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 is no requirement to test terrestrial amphibians or reptiles and there is also no guidance available on how to conduct risk assessments for these groups.

In the absence of toxicity data on fluxapyroxad and azoxystrobin, the active substances in the formulation BAS 736 00 F, and considering the lack of guidance for risk assessment, it is assumed that the risk assessments for birds and mammals are protective for terrestrial life-stages of amphibians and reptiles, an approach that is also used by US-EPA (2004).

References

US-EPA 2004. Overview of the ecological risk assessment process in the Office of Pesticide Programs, U.S. Environmental Protection Agency. Endangered and Threatened Species Effects Determinations. Office of Prevention, Pesticides and Toxic Substances; Office of Pesticide Programs, Washington, D.C. 92 pp.

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

The standard risk assessment for the active substances fluxapyroxad and azoxystrobin indicate an acceptable risk for all groups of aquatic organisms following the intended uses of BAS 736 00 F with no need for additional any mitigation measures.

The PEC/RAC ratios for the relevant metabolites of fluxapyroxad and azoxystrobin are significantly below the trigger of 1 based on standard worst-case assumptions; they are thus considered not to be of ecotoxicological relevance.

The formulation risk assessment revealed an acceptable risk to aquatic organisms following the intended uses of BAS 736 00 F in ‘~~winter and~~ spring cereals’ without the need for any mitigation measures.

For intended uses of BAS 736 00 F in winter cereals, the mitigation measures are required only for scenario R3 (10 m no spray buffer zone including a 10 m vegetated buffer strip).

**The standard risk assessment for the fungicidal product BAS 736 00 F, the active substances fluxapyroxad and azoxystrobin** **as well as their major metabolites demonstrates that the application of BAS 736 00 F in ’winter and spring cereals’ according to good agricultural practice is of low risk to aquatic ecosystems.**

#### Effects on bees (KCP 10.3.1)

The risk to honey bees from the use of fluxapyroxad, azoxystrobin and BAS 736 00 F 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) [*OEPP/EPPO, 2010: Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10 (3), Bulletin OEPP/EPPO Bulletin 40, 323–331*]. Furthermore, under Regulation (EC) No 1107/2009, no risk assessment scheme exists currently for chronic honey bee or honey bee larvae studies. In the absence of clear guidance (noted and agreed by member states) a preliminary risk assessment according to the current legal requirements (SANCO/10329/2002 and EPPO 2010) has been conducted.

The hazard quotients for BAS 736 00 F and the active substances fluxapyroxad and azoxystrobin for acute oral and acute contact exposure of honey bees are considerably below the Commission Regulation (EU) 546/2011 trigger value of 50. Additionally, the chronic TER for larvae and adult bees exceed the suggested trigger. Considering the very protective assumptions the risk can be considered acceptable.

**Based on these results it can be concluded that low risk to honey bees is expected from applications of BAS 736 00 F according to the proposed uses.** **This is confirmed by a worst case assessment following EPPO (2010) for chronic adult and honey bee larvae.**

#### 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 736 00 F is based on Tier I tests with the standard test species *T*. *pyri* and *A*. *rhopalosiphi* and Tier II tests on *T*. *pyri,* *A. rhopalosiphi* and *C. carnea*. The risk assessment is based on the worst-case application rate according to the proposed use pattern.

**Based on the results of the conducted first and higher tier risk assessments it can be concluded that low risk for non-target arthropods is expected from the use of BAS 736 00 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.**

#### 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 and other non-target soil organisms (meso- and macrofauna), as well as for 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***

The potential risk of BAS 736 00 F, fluxapyroxad, azoxystrobin and the relevant metabolites to earthworms and other non-target soil macro-organisms was assessed by comparing the maximum PECsoil values with NOEC or EC10 values, to generate TER values.

**All TER values for BAS 736 00 F, fluxapyroxad, azoxystrobin and the relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the relevant trigger value. This indicates that BAS 736 00 F poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use rate.**

***Effects on soil microbial activity***

The potential risk of BAS 736 00 F, fluxapyroxad, azoxystrobin and the relevant metabolites to soil micro-organisms was assessed by comparing the maximum PECsoil values with the maximum concentration with effects ≤ 25%.

**For the formulation BAS 736 00 F, the active substances fluxapyroxad and azoxystrobin as well as their relevant metabolites, the maximum concentration with effects < 25% (SANCO/10329/2002 trigger) are all above the maximum PECsoil values. Therefore, it is concluded that the use of BAS 736 00 F will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.**

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

The toxicity of BAS 736 00 F to non-target terrestrial plants has been investigated by carrying out vegetative vigor and seedling emergence studies with up to six dicotyledonous and four monocotyledonous non-target plant species. Plants showed similar/higher sensitivity to pre- emergence exposure than to post-emergence exposure. The risk assessment is thus carried out with the respective most sensitive endpoints obtained from the vegetative vigor tests.

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field areas, as non-target plants are non-crop plants located outside the treated area. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates in Appendix IV of ESCORT 2. For a single application to field crops and vegetables < 50 cm, 2.77% of the application rate was assumed to reach areas at 1 m from the edge of the crop (worst-case scenario). The highest single application rate of BAS 736 00 F is used to calculate the maximum off-field predicted environmental rate (PERoff-field). The potential risk of BAS 736 00 F to non-target plants was assessed by comparing the calculated PER value to the ER50 values in order to generate TER values (TER).

Based on the results of the greenhouse trials, all the TER values were above the standard trigger of 5.

**Based on the risk assessment it can be concluded that BAS 736 00 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 736 00 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 736 00 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 and mammals | Application rate | All intended uses | EFSA crop group: Cereals | Maximum worst-case application rate = 2 x 2.0 L/ha (corresponding to 2 x 0.100 kg fluxapyroxad/ha and 2 x 0.150 kg azoxystrobin/ha) |
| Aquatic organisms | Grouping according to Section 8 – Environmental Fate | | | |
| Bees, non-target plants | Application rate | All intended uses | Risk assessments are based on the maximum single application rate of 2 x 2.0 L/ha (corresponding to 2 x 0.100 kg fluxapyroxad/ha and 2 x 0.150 kg azoxystrobin/ha), which covers all other intended uses | Maximum single application rate = 2.0 L/ha (corresponding to 0.100 kg fluxapyroxad/ha and 0.150 kg azoxystrobin/ha) |
| Non-target arthropods | Application rate | All intended uses | Risk assessments are based on the maximum application rate of 2 x 2.0 L/ha (corresponding to 2 x 0.100 kg fluxapyroxad/ha and 2 x 0.150 kg azoxystrobin/ha), which covers all other intended uses | Maximum worst-case application rate = 2 x 2.0 L/ha (corresponding to 2 x 0.100 kg fluxapyroxad/ha and 2 x 0.150 kg azoxystrobin/ha) |
| Soil macro- and micro-organisms | Worst case PECsoil value | All intended uses | Risk assessment is based on the worst case PECsoil value derived from maximum application rate of 2 x 2.0 L/ha (corresponding to 2 x 0.100 kg fluxapyroxad/ha and 2 x 0.150 kg azoxystrobin/ha), which covers all other intended uses | Maximum worst-case application rate = 2 x 2.0 L/ha (corresponding to 2 x 0.100 kg fluxapyroxad/ha and 2 x 0.150 kg azoxystrobin/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 736 00 F is indicated in the table. Metabolites relevant in other areas than soil and aquatics will be dealt in the respective parts of this dossier.

Table 9.1‑3 Metabolites of fluxapyroxad

| Metabolite | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
| --- | --- | --- | --- | --- |
| M700F001 Reg. No. 5069089 |  | 176.1 | Soil: 12.1%  Groundwater: 100%  Water: 10.9%  (not found in sediment)  Air: - | **Terrestrial** Metabolite relevant for RA: yes RA conducted: yes **Aquatic**  Metabolite relevant for RA: yes RA conducted: yes |
| M700F002 Reg. No. 5435595 |  | 162.0 | Soil: 70.5%  Groundwater: 100%  Water/sediment: -  Air: - | **Terrestrial** Metabolite relevant for RA: yes RA conducted: yes  **Aquatic**  Metabolite relevant for RA: yes RA conducted: yes |
| M700F007 Reg. No. 5621781 |  | 175.1 | Soil: -  Groundwater: -  Water: 17.7%  (not found in sediment)  Air: - | **Terrestrial** Metabolite relevant for RA: no RA conducted: no  **Aquatic**  Metabolite relevant for RA: yes RA conducted: yes |

Table 9.1‑4 Metabolites of azoxystrobin

| Metabolite | Chemical structure | Molar mass | Maximum occurrence in compartments | Risk assessment required? |
| --- | --- | --- | --- | --- |
| R234886 |  | 389.4 | > 10 % of a.s. in soil  > 10 % of a.s. in water  > 10 % of a.s. in sediment | Soil: yes  Surface water: yes  Sediment: no\* |
| R401553 |  | 213.2 | > 10 % of a.s. in soil  > 5 % of as in 2 sequential measurements in water / sediment | Soil: yes  Surface water: yes  Sediment: no |
| R402173 |  | 333.3 | > 10 % of a.s. in soil  < 5 % of as in water / sediment | Soil: yes  Surface water: yes  Sediment: no\* |

\* The trigger of 10% is met, however no risk assessment is required for any of these metabolites, because they are not relevant.

## Effects on birds (KCP 10.1.1)

The risk assessment for birds is carried out following the latest guidance document by EFSA (*Anonymous 2009: Guidance Document on risk assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. European Food Safety Authority*), hereafter cited as EFSA/2009/1438.

### Toxicity data

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

**Active substances**

An overview of the EU agreed endpoints is given in Table 9.2‑1 (fluxapyroxad) and Table 9.2‑2 (azoxystrobin). 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 9.2‑1: Fluxapyroxad (BAS 700 F): Endpoints relevant for the risk assessment for birds

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Substance** | **Exposure**  **System** | **Results** | **Reference**  **[BASF DocID]** |
| *Colinus virginianus* | Fluxapyroxad | Oral, 1 d Acute | LD50 > 2000 mg a.s./kg b.w. | EFSA Journal 2012;10(1):2522  [2007/1054365] |
| *Anas platyrhynchos* | Fluxapyroxad | Oral, 1 d Acute | LD50 > 2000 mg a.s./kg b.w. | EFSA Journal 2012;10(1):2522  [2008/1003797] |
| *Taeniopygia guttata* | Fluxapyroxad | Oral, 1 d Acute | LD50 > 2000 mg a.s./kg b.w. | New study  [2009/1050338] |
| *Colinus virginianus* | Fluxapyroxad | Dietary Reproductive toxicity | NOEL ≥ 74.6 mg a.s./kg b.w./d 1) 2) | EFSA Journal 2012;10(1):2522  [2008/1023021 and 2009/1079886 (amendment no.1), 2009/1099679 (amendment no.2)] |
| *Anas platyrhynchos* | Fluxapyroxad | Dietary Reproductive toxicity | NOEL = 33.6 mg a.s./kg b.w./d 1) 2) | EFSA Journal 2012;10(1):2522  [2008/1055153 and 2009/1079887 (amendment no.1)] |
| **Endpoint used for acute assessment** | **Fluxapyroxad** | **Oral, 1 d**  **Acute** | **LD50 (extrapolated, geometric mean) = 3776 mg/kg b.w. 3)** | **Extrapolation and geometric mean of quail, mallard and zebra finch LD50**  **[2007/1054365, 2008/1003797, 2009/1050338]** |
| **Endpoint used for reproductive assessment** | **Fluxapyroxad** | **Dietary Reproductive toxicity – Tier 1** | **NOEL = 33.6 mg a.s./kg b.w./d 1) 2)** | **EFSA Journal 2012;10(1):2522**  **[2008/1055153 and 2009/1079887 (amendment no.1)]** |

1) The Reproductive endpoints as calculated by the notifier for *Colinus virginianus* and *Anas platyrhynchos* are slightly are slightly different to the ones as derived during the Annex I inclusion process based on the same two studies.

2) Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight.

3) For details please refer to chapter 9.2.1.1.

Table 9.2‑2: Azoxystrobin: Endpoints relevant for the risk assessment for birds

| Species | Substance | Exposure  System | Results | Reference  [Study ID] |
| --- | --- | --- | --- | --- |
| Bobwhite quail (*Colinus virginianus*) | Azoxystrobin | Oral 1 d Acute | LD50 > 2000 mg/kg b.w. | EFSA Journal 2010; 8(4):1542 [ICI5504/0852] |
| Bobwhite quail (*Colinus virginianus*) | Azoxystrobin | Dietary 5 d Short-term | LDD50 > 1179 1) mg/kg b.w./d | EFSA Journal 2010; 8(4):1542  [ICI5504/0854] |
| Bobwhite quail (*Colinus virginianus*) | Azoxystrobin | Dietary Reproductive toxicity | NOEL = 117 1) mg/kg b.w./d | EFSA Journal 2010; 8(4):1542  [ICI5504/0855] |
| **Endpoint used for acute assessment** | **Azoxystrobin** | **Oral 1 d Acute** | **LD50 > 2000 mg/kg b.w.** | **EFSA Journal 2010; 8(4):1542 [ICI5504/0852]** |
| **Endpoint used for reproductive assessment** | **Azoxystrobin** | **Dietary Reproductive toxicity** | **NOEL = 117 1) mg/kg b.w./d** | **EFSA Journal 2010; 8(4):1542 [ICI5504/0855]** |

1) re-calculated endpoints in terms of daily dietary dose; it is the belief of the applicant that the units are erroneously displayed in the EFSA Conclusion (2010); 8(4):1542.

**Metabolites**

**Metabolites of fluxapyroxad**

In the assessment of the metabolites of fluxapyroxad carried out in the EU evaluation of this active substance (EFSA (2012) Scientific report 10 (1) 2522: Conclusion on the peer review of fluxapyroxad (BAS 700 F), it was concluded that no specific risk assessment for birds and mammals for any of the fluxapyroxad metabolites is necessary. Therefore, no risk assessment for metabolites is presented in this dossier.

**Metabolites of azoxystrobin**

In the assessment of the metabolites of azoxystrobin carried out in the EU evaluation of this active substance (EFSA (2010) Scientific report 8 (4) 1542: Conclusion on the peer review of azoxystrobin, it was concluded that no specific risk assessment for birds and mammals for any of the azoxystrobin metabolites is necessary. Therefore, no risk assessment for metabolites is presented in this dossier.

**Formulation toxicity**

An acute toxicity test in bobwhite quails has been carried out with the formulation BAS 736 00 F (BASF DocID 2020/2095640; see section 9: Appendix 2.1.1), as required by EU Commission Regulation No. 284/2013 due to the acute toxicity of the formulation to rats. No mortality occurred in this study, with LD50 > 2000 mg formulation/kg b.w.. This indicates low toxicity of the formulation and no increased toxicity of the formulation compared to the active substances. Consequently, the acute risk from the formulation is covered by the acute risk assessment for the active substances.

#### Justification for new endpoints

**Fluxapyroxad**

Acute – Because no mortality occurred in the acute oral gavage studies in quail (BASF DocID 2007/1054365), mallard (BASF DocID 2008/1003797) and zebra finch (BASF DocID 2009/1050338), the endpoints from each study (LD50 > 2000 mg/kg b.w.) were extrapolated to LD50 (extrapolated) = 3 776 mg/kg b.w. Determination of the geometric mean out of the three extrapolated LD50 values resulted in an LD50 (extrapolated, geometric mean) = 3 776 mg a.s./kg b.w. of the acute oral toxicity studies.

|  |
| --- |
| **Review Comments:**  At the screening step LD50 value of >2000 mg a.s./kg bw will be used in the risk assessment. |

Reproductive - Not applicable. Endpoint is EU agreed.

**Azoxystrobin**

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed

**Formulation toxicity**

An acute toxicity test in bobwhite quails has been carried out with the formulation BAS 736 00 F

### Risk assessment for spray applications

**Proposed use pattern for the risk assessments**

The proposed use pattern for the use of BAS 736 00 F is summarized in Table 9.2‑3. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

Table 9.2‑3: Proposed use pattern

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Crop** | **Crop group according to EFSA/2009/1438** | **Application time**  **(BBCH growth stage)** | **Number of applications** | **Interval between applications**  **[d]** | **Max. application rate per application** | | |
| **Fluxapyroxad [kg/ha]** | **Azoxystrobin**  **[kg/ha]** | **BAS 736 00 F [L/ha]** |
| Barley, Oat, Rye, Triticale, Wheat | Cereals | 30-69 | 1-2 | 21 | 0.100 | 0.150 | ~~1.0-~~2.0 |

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

The dietary TER acute (TERA) and reproductive (TERLT) values for the screening step and tier 1 risk assessment were calculated with the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) according to EFSA/2009/1438.

**Dietary risk assessment for the active substances**

**Acute risk assessment**

The TERA values are presented in Table 9.2‑4 for fluxapyroxad and in Table 9.2‑5 for azoxystrobin. All the TERA values at the screening step are above the relevant trigger of 10 for acceptability of acute effects.

Table 9.2‑4: Fluxapyroxad: Screening step calculation of the acute risk for birds due to the use of BAS 736 00 F for the crop group “cereals”

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | **DT50** | **LD50** |  |
| Cereals | 0.100 | 2 | 21 | 10.0 | ~~3776.0~~  >2000 |  |
| **Screening step:** | | | | | | | |
| Acute risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF (90)** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step required |
| Small omnivorous bird | 158.8 | 15.88 | 1.1 | 17.47 | ~~216.2~~  >114.5 |

Table 9.2‑5: Azoxystrobin: Screening step calculation of the acute risk for birds due to the use of BAS 736 00 F for the crop group “cereals”

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | **DT50** | **LD50** |  |
| Cereals | 0.150 | 2 | 21 | 10.0 | > 2000 |  |
| **Screening step:** | | | | | | | |
| Acute risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF (90)** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step required |
| Small omnivorous bird | 158.8 | 23.82 | 1.1 | 26.20 | > 76.3 |

**Reproductive risk assessment**

The dietary TER reproductive values are presented in Table 9.2‑6 for fluxapyroxad and in Table 9.2‑7

for azoxystrobin. All the TERLT values at the screening step are above the relevant trigger of 5 for acceptability of reproductive effects.

**Table 9.2‑6: Fluxapyroxad: Screening step calculation of the long-term/reproductive risk for birds due to the use of BAS 736 00 F for the crop group “cereals”**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | **DT50** | **Reproductive End Point (mg/kg b.w./d)** | **Time weighted average (TWA)** |
| Cereals | 0.100 | 2 | 21 | 10 | 33.6 | 0.53 |
| **Screening step:** | | | | | | | |
| Reproductive risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF mean** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step required |
| Small omnivorous bird | 64.8 | 6.48 | 1.2 | 4.12 | ~~8.2~~ 8.1 |

**Table 9.2‑7: Azoxystrobin: Screening step calculation of the long-term/reproductive risk for birds due to the use of BAS 736 00 F for the crop group “cereals”**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | **DT50** | **Reproductive End Point (mg/kg b.w./d)** | **Time weighted average (TWA)** |
| Cereals | 0.150 | 2 | 21 | 10 | 117.0 | 0.53 |
| **Screening step:** | | | | | | | |
| Reproductive risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF mean** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step  required |
| Small omnivorous bird | 64.8 | 9.72 | 1.2 | 6.18 | 18.9 |

The conclusions for the dietary risk assessments for each of the active substances are as follows: acceptable acute and reproductive risks for birds were shown at the screening levels for both fluxapyroxad and azoxystrobin. No higher-tier dietary risk assessments are necessary.

**Dietary risk assessment for combined effects of simultaneous exposure to several active substances**

**Combined acute toxicity**

According to EFSA/2009/1438 section 2.5, this assessment is relevant for BAS 736 00 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD50 ~~= 2463.5~~ > 2000 mg/kg b.w. is calculated based on the assumption of dose additivity (). A combined acute risk assessment is not required if for one active substance the deviation between ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ is ≤ 10% as in that case the risk is covered by the assessment for that active substance. For BAS 736 00 F this does not apply because the deviation for both active substances is more than 10% (Table 9.2‑8).

**Table 9.2‑8: Calculation of surrogate LD50 for the mixture of active substances**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Active**  **substance** | **Concentration a.s. in mixture**  **[g/L]** | **Fraction a.s. in mixture** | **LD50 a.s.**  **[mg/kg b.w.]** | **Fraction a.s./**  **LD50 a.s.** | **Surrogate LD50**  **[mg/kg b.w.]** | **Tox per fraction (a.s.)** | **Deviation tox per fraction (a.s.) and tox per fraction (mix)[%]** |
| Fluxapyroxad | 50 | 0.4 | ~~3776~~  > 2000 | ~~0.00011~~  0.00020 | **~~2463.5~~**  **>2000** | ~~9440~~  5000 | ~~283~~ 150 |
| Azoxystrobin | 75 | 0.6 | > 2000 | 0.00030 | 3333.3 | ~~35~~ 67 |

A laboratory study on the acute toxicity of formulation BAS 736 00 F to birds (BASF DocID 2020/2095640) has been conducted and resulted in an acute LD50 > 2000 mg/kg b.w. (see 9.2.1).

Appendix B of EFSA/2009/1438 recommends comparing the surrogate LD50 with the experimental LD50 from formulation testing and running the risk assessment with the lowest of the two values. However, Appendix B does not provide clear recommendations if, for the comparison of the two LD50 values and for the calculation of the exposure scenarios, only the content of the active substances should be considered as the surrogate LD50 is based on toxicity and concentration of active substances, while the experimental LD50 is based on all components of the formulation. Due to this lack of guidance in Appendix B the most comprehensive approach is adopted by the notifier by presenting the two possible risk assessments, one for the virtual compound and another for the formulation.

***Exposure and acute risk assessment for the combined active substances (virtual compound approach)***

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum single application rate of formulation BAS 736 00 F is 2.0 L product/ha (corresponding to 0.1 kg/ha fluxapyroxad and 0.15 kg/ha azoxystrobin for the use in cereals); applying the concept for dose additivity to the exposure calculations results in a combined application rate of 0.25 kg virtual compound/ha.

The dietary TER acute value for the screening step presented in Table 9.2‑9 is above the trigger of 10. Therefore, the acute risk to birds from combined effects of the two active substances in BAS 736 00 F is acceptable.

**Table 9.2‑9: Screening step calculation of the acute risk for birds due to the use of BAS 736 00 F in the crop group “cereals” – virtual compound approach**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg virtual compound/ha)** | **Number of applications** | **Application Interval** | **DT50** | **LD50** |  |
| Cereals | 0.250 | 2 | 21 | 10.0 | ~~2463.5~~  2000 |  |
| **Screening step:** | | | | | | | |
| Acute risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF (90)** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step required |
| Small omnivorous bird | 158.8 | 39.70 | 1.1 | 43.67 | ~~56.4~~  45.8 |

Note that this virtual compound acute TER was calculated according to the concentration addition approach and thus gives the same value as if calculated using equation TERA combi = trigger/((trigger/TERA substance 1)+(trigger/TERA substance 2)).

***Exposure and acute risk assessment for combined active substances (formulation approach)***

BAS 736 00 F is intended to be used with a maximum single application rate of 2.0 L product/ha in the crop group cereals. Taking into account the density of the formulation of 1.078 g/cm3, this will result in an application rate of 2.156 kg BAS 736 00 F/ha.

The dietary TER acute values for the tier 1 risk assessment presented in Table 9.2‑10 are above the trigger of 10. Therefore, the acute risk to birds from exposure to BAS 736 00 F is acceptable.

**Table 9.2‑10: Screening step and tier 1 calculations of the acute risk for birds due to the use of BAS 736 00 F in the crop group “cereals” – formulation approach**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | | **Crop** | **Application rate (kg formulation/ha)** | | **Number of applications** | **Application Interval** | **DT50** | | **LD50** |  |
| Cereals | 2.156 | | 2 | 21 | 10.0 | | > 2000 |  |
| **Screening step:** | | | | | | | | | | |
| Acute risk assessment screening step | | **Indicator species** | **Shortcut value** | | **Daily Dietary Dose (single)** | **MAF (90)** | **Daily Dietary Dose (Multiple)** | | **TER** | **Please perform first tier risk assessment (see below)** |
| Small omnivorous bird | 158.8 | | 342.37 | 1.1 | 376.61 | | **> 5.3** |
| **First Tier Risk Assessment:** | | | | | | | | | | |
| Calculate TER for each generic focal species | **Crop** | | | **Generic focal species** | | | **Short cut value** | **TER** | |  |
| Cereals BBCH 30 -39 | | | Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods | | | 12.0 | > 70.3 | | No refinement  required |
| Cereals BBCH ≥ 40 | | | Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods | | | 7.2 | > 117.1 | |

TER values in **bold** are below the trigger.

In conclusion, the two risk assessment approaches (combined toxicity of the active substances and formulation toxicity) have resulted in acute TER values that are above the trigger of 10 for acceptability of effects at the screening step and/or tier 1 risk assessment. Therefore, the acute dietary risk to birds from BAS 736 00 F is acceptable.

**Combined reproductive toxicity**

As requested in the summary report of the Steering Committee of the Central Zone Harmonisation workshop in April 2015 and update of October 2016 (Central Zone Harmonisation Workshop, 2016), a long-term combination toxicity tier 1 risk assessment is presented. As proposed there, the calculations follow the concentration addition model.

The combined TERLT value is calculated according to the following formula:

TERLT combi = trigger/((trigger/TERLT substance 1)+(trigger/TERLT substance 2))

An acceptable risk is expected when TERLT combi > trigger.

The TERLT combi values are calculated based on screening step TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in Table 9.2‑11.

**Table 9.2‑11:** **Combined reproductive toxicity risk assessment for birds due to the use of BAS 736 00 F for the crop group “cereals”**

| **Crop scenario and/or indicator species** | | **TERLT 1)**  **fluxapyroxad** | **TERLT 1)**  **azoxystrobin** | **TERLT combi** | **Trigger** |
| --- | --- | --- | --- | --- | --- |
| **Reproductive (screening step)** | | | | | |
| Cereals | Small omnivorous bird | ~~8.2~~ 8.1 | 18.9 | 5.7 | 5 |

1) Reproductive TER values are presented in Table 9.2‑6 (fluxapyroxad) and Table 9.2‑7 (azoxystrobin).

The TERLT combi value in the screening step is above the trigger value of 5. Thus, it can be concluded that the reproductive risk for birds for the combined exposure to the two active substances in the application of BAS 736 00 F according to good agricultural practice is low and acceptable.

#### Higher-tier risk assessment

Not necessary as acceptable acute and reproductive risks were shown with the screening and/or first-tier risk assessments for all scenarios.

#### Drinking water exposure

Leaf scenario

Since BAS 736 00 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 b.w./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).

The ratio calculations for effective application rate to relevant endpoint are detailed in Table 9.2‑12 (fluxapyroxad) and Table 9.2‑13 (azoxystrobin). The ratios for acute and reproductive endpoints for fluxapyroxad (0.05 and 5.68, respectively) and for azoxystrobin (< 0.14 and 2.35, respectively) do not exceed the threshold value of 3000 and 50, respectively, 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 9.2‑12: Assessment of the risk for birds due to exposure to fluxapyroxad via contaminated drinking water in puddles**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Fluxapyroxad** | **Reference** |
| Kfoc (geometric mean) [L/kg] | 681 | Chapter 8.5.1 |
| DT50 (soil) [days] (geometric mean) | 151 | Chapter 8.9 |
| Number of applications | 2 | Chapter 9.1 |
| Interval [days] | 21 | Chapter 9.1 |
| MAFm 1) | 1.91 | -- |
| Max use rate [g/ha] | 100.0 | Chapter 9.1 |
| AReff [g/ha] 2) | 191.0 | -- |
| LD50 [mg/kg b.w.] | ~~3776~~ >2000 | Chapter 9.2.1 |
| Ratio (acute) 3) | ~~0.05~~ 0.096 | -- |
| NO(A)EL [mg/kg b.w./d] | 33.6 | Chapter 9.2.1 |
| Ratio (repro) 3) | 5.68 | -- |
| 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 MAFm

3) Ratio of AReff and relevant toxicity endpoint

**Table 9.2‑13: Assessment of the risk for birds due to exposure to azoxystrobin via contaminated drinking water in puddles**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Azoxystrobin** | **Reference** |
| Koc (geometric mean) [L/kg] | 392 | Chapter 8.5.1 |
| DT50 (soil) (geometric mean) [days] | 78 | Chapter 8.9 |
| Number of applications | 2 | Chapter 9.1 |
| Interval [days] | 21 | Chapter 9.1 |
| MAFm 1) | 1.83 | -- |
| Max use rate [g/ha] | 150.0 | Chapter 9.1 |
| AReff [g/ha] 2) | 274.5 | -- |
| LD50 [mg/kg b.w.] | > 2000 | Chapter 9.2.1 |
| Ratio (acute) 3) | < 0.14 | -- |
| NO(A)EL [mg/kg b.w./d] | 117.0 | Chapter 9.2.1 |
| Ratio (repro) 3) | 2.35 | -- |
| Trigger | 50 | -- |
| 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 MAFm

3) Ratio of AReff and relevant toxicity endpoint

In conclusion, the risk to birds via drinking water from the intended use of BAS 736 00 F according to the proposed use pattern is acceptable.

#### Effects of secondary poisoning

The log Pow of the active substance fluxapyroxad is 3.1 (BASF DocID 2007/1057001), which triggers an assessment of the potential risk from secondary poisoning.

The log Pow of the active substance azoxystrobin is 2.5 (EFSA Journal 2010; 8(4): 1542) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

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.

As shown in the following Table 9.2‑14 the TERLT for fluxapyroxad exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning.

**Table 9.2‑14: Assessment of the risk for earthworm-eating birds due to exposure to fluxapyroxad via bioaccumulation in earthworms (secondary poisoning) for the intended use**

| **Parameter** | **Fluxapyroxad** | **Reference** |
| --- | --- | --- |
| PECsoil (accu) [mg/kg soil] 1) | 0.078 | Chapter 8.7 |
| Kow | 1259 | BASF DocID 2007/1057001 |
| Koc (geometric mean) [L/kg] | 681 | Chapter 8.5.1 |
| foc (default) | 0.02 | EFSA/2009/1438 |
| BCF 2) | 1.171 | -- |
| PECworm [mg/kg] 3) | 0.091 | -- |
| Daily dose [mg/kg b.w./d] 4) | 0.096 | -- |
| NO(A)EL [mg/kg b.w./d] | 33.6 | Chapter 9.2.1 |
| TERlt 5) | 350.4 | -- |

1) Worst case PECsoil (accu) value was calculated for an application scenario of 2 x 100 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

2) Bioconcentration factor (BCF) = (0.84 + 0.012 x Kow) / (foc x Koc)

3) PECworm = PECsoil x BCF

4) Daily dose = 1.05 x PECworm

5) TERLT = NO(A)EL / Daily dose.

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 9.2‑15, the TERLT for fluxapyroxad exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating birds via secondary poisoning.

**Table 9.2‑15: Assessment of the risk for fish-eating birds due to exposure to fluxapyroxad via bioaccumulation in fish (secondary poisoning) for the intended use**

| **Parameter** | **Fluxapyroxad** | **Reference** |
| --- | --- | --- |
| PECsw (twa, 21 d) [mg/L] 1) | 6.123 × 10-3 | Chapter 8.9 (Step 2, Northern Europe) |
| BCFfish (max. worst case) | 36 | EFSA Journal 2012; 10(1): 2522 |
| PECfish [mg/kg] 2) | 0.220 | -- |
| Daily dose [mg/kg b.w./d] 3) | 0.035 | -- |
| NO(A)EL [mg/kg b.w./d] | 33.6 | Chapter 9.2.1 |
| TERlt 4) | 958.7 | -- |

1) PECsw (twa, 21 d) value calculated for a multiple application scenario of 2 x 100 g a.s./ha to cereals from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

2) PECfish = PECsw (twa, 21 d) x BCF

3) Daily dose = 0.159 x PECfish

4) TERLT = NO(A)EL / Daily dose.

#### Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU review of fluxapyroxad (EFSA Journal 2012; 10(1): 2522).

No evidence of accumulation in animal tissue was concluded in the EU review of azoxystrobin from toxicokinetics studies (EFSA Journal 2010; 8(4): 1542).

Since the bioaccumulation potential of both fluxapyroxad and azoxystrobin is low no further assessment on biomagnification 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 736 00 F according to good agricultural practice is acceptable.**

|  |
| --- |
| **Review Comments:**  The acute and chronic risks ofBAS 736 00 F to 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 736 00 F does 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 potential risk of secondary poisoning is low. |

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

The risk assessment for mammals is carried out following the latest guidance document by EFSA (EFSA/2009/1438).

### Toxicity data

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

**Active substances**

The selection of studies and endpoints for the risk assessment of fluxapyroxad (Table 9.3‑1) and azoxystrobin (Table 9.3‑2) is in line with the results of the EU review process.

Table 9.3‑1: Fluxapyroxad (BAS 700 F): Endpoints relevant for the risk assessment for mammals

| **Species** | **Substance** | **Exposure**  **System** | **Results** | **Reference**  **[BASF DocID]** |
| --- | --- | --- | --- | --- |
| Rat | Fluxapyroxad | Oral, 1 d Acute | LD50 > 2000 mg a.s./kg b.w. | EFSA Journal 2012;10(1):2522  [2008/1002441] |
| Rat | Fluxapyroxad | Dietary Reproductive toxicity Two-generation study | NOAEL = 10 mg a.s./kg b.w./d 1) | EFSA Journal 2012;10(1):2522  [2009/1072491] |
| Rat | Fluxapyroxad | Oral Prenatal Developmental toxicity | NOAEL maternal tox = 25 mg a.s./kg b.w./d 1)  NOAEL developmental tox = 1000 mg a.s./kg b.w./d 1) | EFSA Journal 2012;10(1):2522  [2009/1072492] |
| Rabbit | Fluxapyroxad | Oral Prenatal Developmental toxicity | NOAEL maternal tox = 25 mg a.s./kg b.w./d1)  NOAEL developmental tox = 25 mg a.s./kg b.w./d1) | EFSA Journal 2012;10(1):2522  [2009/1072493] |
| **Endpoint used for acute risk assessment** | **Fluxapyroxad** | **Oral, 1 d Acute** | **LD50 > 2000 mg a.s./kg b.w.** | **EFSA Journal 2012;10(1):2522**  **[2008/1002441]** |
| **Endpoint used for reproductive risk assessment** | **Fluxapyroxad** | **Dietary Reproductive toxicity Two-generation study** | **NOAEL = 10 mg a.s./kg b.w./d 1)** | **EFSA Journal 2012;10(1):2522**  **[2009/1072491]** |

1) Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight.

Table 9.3‑2: Azoxystrobin: Endpoints and effect values relevant for the risk assessment for mammals

| Species | Substance | Exposure  System | Results | Reference  [Study ID] |
| --- | --- | --- | --- | --- |
| Rat | Azoxystrobin | Oral 1 d Acute | LD50 > 5000 mg a.s./kg b.w. | EFSA Journal 2010; 8(4):1542 [ICI5504/0081] |
| Rat | Azoxystrobin | Dietary Reproductive toxicity Two-generation study | NOAEL = 32 mg a.s./kg b.w./d  (offspring effects on pup weight) | EFSA Journal 2010; 8(4):1542  [ICI5504/0117] |
| **Endpoint used for acute risk assessment** | **Azoxystrobin** | **Oral 1 d Acute** | **LD50 > 5000 mg a.s./kg b.w.** | **EFSA Journal 2010; 8(4):1542**  **[ICI5504/0081]** |
| **Endpoint used for reproductive risk assessment** | **Azoxystrobin** | **Dietary Reproductive**  **toxicity Two-generation study** | **NOAEL = 32 mg a.s./kg b.w./d** | **EFSA Journal 2010; 8(4):1542**  **[ICI5504/0117]** |

**Metabolites**

**Metabolites of fluxapyroxad**

See section 9.2.1 in the bird chapter.

**Metabolites of azoxystrobin**

See section 9.2.1 in the bird chapter.

**Formulation toxicity**

For toxicological classification and labeling purposes, an acute oral toxicity study with BAS 736 00 F in rats was carried out according to the toxic class method described in OECD 423 (BASF DocID 2020/2080214; see chapter 6.3 and Appendix 2 of chapter 6). No mortality occurred in 6 animals dosed with 500 mg formulation/kg b.w., while two out of three animals dosed with 2000 mg formulation/kg b.w. died, resulting in LD50 > 500 and < 2000 mg formulation/kg b.w..

In mammalian toxicology assessment, the dose-response curve was fitted via the probit model according to Finney to estimate a bound LD50 for BAS 736 00 F (see chapter 6.3 and Appendix 2 of chapter 6). This interpolated LD50 value of 1848 mg formulation/kg b.w. is additionally considered in refined risk assessment.

As an alternative approach a cut-off LD50 value can be determined following Annex 2d of OECD Test 423 (see figure 1 at the end of the commenting table). In a 1st step, a cut-off LD50 of 1000 mg/kg bw can be estimated according to Annex 2d: 2/3 animals died at 2000 mg and no animals (2 x 3) died at 500 mg/kg bw. In a 2nd step a pro-rata dose correction of the cut-off LD50 is deemed to be reasonable resulting in a cut-off LD50 of = 1667 mg/kg bw (500/300 x 1000). In this study 500 mg/kg bw were tested instead of 300 mg/kg bw, but no mortality was detected for 2 x 3 animals at the lower dose level. Therefore, it can be assumed that the cut-off LD50 of 1000 mg/kg would be overly conservative. A LD50 of 1667 mg/kg bw is further supported by the fact that 2 of 3 animals = 66% died at 2000 mg/kg bw, which indicates that the ‘true’ LD50 is likely to be close to 2000 mg/kg bw.

The ‘greater than’ LD50 of 500 mg formulation/kg b.w. is used for risk assessment as worst-case.

#### Justification for new endpoints

**Fluxapyroxad**

Acute - Not applicable. Endpoint is EU agreed.

Reproductive - Not applicable. Endpoint is EU agreed.

**Azoxystrobin**

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

**Formulation toxicity**

An acute oral toxicity study with BAS 736 00 F in rats was carried out and used in the risk assessment.

### Risk assessment for spray applications

**Proposed use pattern for the risk assessments**

The proposed use pattern for the use of BAS 736 00 F is summarized in Table 9.3‑3. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

**Table 9.3‑3: Proposed use pattern**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Crop** | **Crop group according to EFSA/2009/1438** | **Application time**  **(BBCH growth stage)** | **Number of applications** | **Interval between applications**  **[d]** | **Max. application rate per application** | | |
| **Fluxapyroxad [kg/ha]** | **Azoxystrobin**  **[kg/ha]** | **BAS 736 00 F [L/ha]** |
| Barley, Oat, Rye, Triticale, Wheat | Cereals | 30-69 | 1-2 | 21 | 0.100 | 0.150 | ~~1.0-~~2.0 |

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

The dietary TER acute (TERA) and reproductive (TERLT) values for the screening step and tier 1 risk assessment were calculated with the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) according to EFSA/2009/1438.

**Dietary risk assessment for the active substances**

**Acute risk assessment**

The TERA values are presented in Table 9.3‑4 for fluxapyroxad and in Table 9.3‑5 for azoxystrobin. All the TERA values at the screening step are above the relevant trigger of 10 for acceptability of acute effects.

**Table 9.3‑4: Fluxapyroxad: Screening step calculation of the acute risk for mammals due to the use of BAS 736 00 F for the crop group “cereals”**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | **DT50** | **LD50** |  |
| Cereals | | 0.100 | 2 | 21 | 10.0 | > 2000.0 |  |
| **Screening step:** | | | | | | | | |
| Acute risk assessment screening step | **Indicator species** | **Shortcut value** | | **Daily Dietary Dose (single)** | **MAF (90)** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step required |
| Small herbivorous mammal | 118.4 | | 11.84 | 1.1 | 13.02 | > 153.6 |

**Table 9.3‑5: Azoxystrobin: Screening step calculation of the acute risk for mammals due to the use of BAS 736 00 F for the crop group “cereals”**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | **DT50** | **LD50** |  |
| Cereals | | 0.150 | 2 | 21 | 10.0 | > 5000.0 |  |
| **Screening step:** | | | | | | | | |
| Acute risk assessment screening step | **Indicator species** | **Shortcut value** | | **Daily Dietary Dose (single)** | **MAF (90)** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step required |
| Small herbivorous mammal | 118.4 | | 17.76 | 1.1 | 19.54 | > 255.9 |

**Reproductive risk assessment**

The dietary TER reproductive values for the screening step and tier 1 risk assessment are presented in Table 9.3‑6 for fluxapyroxad and in Table 9.3‑7 for azoxystrobin. All the TERLT values at the screening step or at tier 1 are above the relevant trigger of 5 for acceptability of reproductive effects.

**Table 9.3‑6: Fluxapyroxad: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 736 00 F for the crop group “cereals”**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | | **DT50** | **Reproductive End Point (mg/kg b.w./d)** | **Time weighted average (TWA)** |
| Cereals | 0.100 | 2 | 21 | | 10 | 10.0 | 0.53 |
| **Screening step:** | | | | | | | | |
| Reproductive risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF mean** | **Daily Dietary Dose (Multiple)** | | **TER** | Please perform first tier risk assessment (see below) |
| Small herbivorous mammal | 48.3 | 4.83 | 1.2 | 3.07 | | **3.26** |
| **First Tier Risk Assessment:** | | | | | | | | |
| Calculate TER for each generic focal species selected | **Crop** | **Generic focal species** | | | **Shortcut value** | | **TER** | No refinement required |
| Cereals BBCH ≥ 20 | Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods | | | 1.9 | | 82.8 |
| Cereals BBCH 30 - 39 | Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods | | | 3.9 | | 40.3 |
| Cereals BBCH ≥ 40 | Small herbivorous mammal "vole Grass + cereals 100% grass | | | 21.7 | | 7.2 |
| Cereals BBCH ≥ 40 | Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods | | | 2.3 | | 68.4 |

TER values in **bold** are below the trigger

**Table 9.3‑7: Azoxystrobin: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 736 00 F for the crop group “cereals”**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg a.s./ha)** | **Number of applications** | **Application Interval** | | **DT50** | **Reproductive End Point (mg/kg b.w./d)** | **Time weighted average (TWA)** |
| Cereals | 0.150 | 2 | 21 | | 10 | 32.0 | 0.53 |
| **Screening step:** | | | | | | | | |
| Reproductive risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF mean** | **Daily Dietary Dose (Multiple)** | | **TER** | No refinement step required |
| Small herbivorous mammal | 48.3 | 7.25 | 1.2 | 4.61 | | 6.94 |
| **First Tier Risk Assessment: 1)** | | | | | | | | |
| Calculate TER for each generic focal species selected | **Crop** | **Generic focal species** | | | **Shortcut value** | | **TER** | No refinement required |
| Cereals BBCH ≥ 20 | Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods | | | 1.9 | | 176.5 |
| Cereals BBCH 30 - 39 | Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods | | | 3.9 | | 86.0 |
| Cereals BBCH ≥ 40 | Small herbivorous mammal "vole Grass + cereals 100% grass | | | 21.7 | | 15.5 |
| Cereals BBCH ≥ 40 | Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods | | | 2.3 | | 145.8 |

1) The screening step resulted in a TER value above the relevant trigger of 5. Hence, a first-tier risk assessment is not necessary. However, first-tier TER calculations are presented as they are required for calculation of combined reproductive toxicity.

The conclusions for the first-tier dietary risk assessments for each of the active substances are as follows: acceptable acute and reproductive risks for mammals were shown at the screening and/or tier 1 levels for both fluxapyroxad and azoxystrobin. No higher-tier dietary risk assessments are necessary.

**Dietary risk assessment for combined effects of simultaneous exposure to several active substances**

**Combined acute toxicity**

According to EFSA/2009/1438 section 2.5 this assessment is relevant for BAS 736 00 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD50 = 3125.0 mg/kg b.w. is calculated based on the assumption of dose additivity (). A combined acute risk assessment is not required if for one active substance the deviation between ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ is ≤ 10% as in that case the risk is covered by the assessment for that active substance. For BAS 736 00 F this does not apply because the deviation for both active substances is more than 10% (Table 9.3‑8).

**Table 9.3‑8: Calculation of surrogate LD50 for the mixture of active substances**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Active substance** | **Concentration a.s. in mixture**  **[g/L]** | **Fraction a.s. in mixture** | **LD50 a.s.**  **[mg/kg b.w.]** | **Fraction a.s./**  **LD50 a.s.** | **Surrogate LD50**  **[mg/kg b.w.]** | **Tox per fraction (a.s.)** | **Deviation tox per fraction (a.s.) and tox per fraction (mix)[%]** |
| Fluxapyroxad | 50 | 0.4 | > 2000 | 0.0002 | **3125.0** | 5000 | 60 |
| Azoxystrobin | 75 | 0.6 | > 5000 | 0.00012 | 8333 | 167 |

A laboratory study on the acute toxicity of formulation BAS 736 00 F to rats (BASF DocID 2020/2080214) resulted in LD50 > 500 and < 2000 mg formulation/kg b.w. (see 9.3.1).

Appendix B of EFSA/2009/1438 recommends comparing the surrogate LD50 with the experimental LD50 from formulation testing and to run the risk assessment with the lowest of the two values. However, Appendix B does not provide clear recommendations if, for the comparison of the two LD50 values and for the calculation of the exposure scenarios, only the content of the active substances should be considered as the surrogate LD50 is based on toxicity and concentration of active substances, while the experimental LD50 is based on all components of the formulation. Due to this lack of guidance in Appendix B the most comprehensive approach is adopted by the notifier by presenting the two possible risk assessments, one for the virtual compound and another for the formulation.

***Exposure and acute risk assessment for combined active substances (virtual compound approach)***

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum single application rate of formulation BAS 736 00 F is 2.0 L product/ha (corresponding to 0.1 kg/ha fluxapyroxad and 0.15 kg/ha azoxystrobin for the use in cereals); applying the concept for dose additivity to the exposure calculations results in a combined application rate of 0.25 kg virtual compound/ha.

The dietary TER acute value for the screening step presented in Table 9.3‑9 is above the trigger of 10. Therefore, the acute risk to mammals from combined effects of the two active substances in BAS 736 00 F is acceptable.

**Table 9.3‑9: Screening step calculation of the acute risk for mammals due to the use of BAS 736 00 F in “cereals” – virtual compound approach**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | **Application rate (kg virtual compound/ha)** | **Number of applications** | **Application Interval** | **DT50** | **LD50** |  |
| Cereals | 0.25 | 2 | 21 | 10.0 | 3125.0 |  |
| **Screening step:** | | | | | | | |
| Acute risk assessment screening step | **Indicator species** | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF (90)** | **Daily Dietary Dose (Multiple)** | **TER** | No refinement step required |
| Small herbivorous mammal | 118.4 | 29.60 | 1.1 | 32.56 | 96.0 |

Note that this virtual compound acute TER was calculated according to the concentration addition approach and thus gives the same value as if calculated using equation TERA combi = trigger/((trigger/TERA substance 1)+(trigger/TERA substance 2)).

***Exposure and acute risk assessment for combined active substances (formulation approach)***

BAS 736 00 F is intended to be used with a maximum single application rate of 2.0 L product/ha in the crop group cereals. Taking into account the density of the formulation of 1.078 g/cm3, this will result in an application rate of 2.156 kg BAS 736 00 F/ha. The ‘greater than’ LD50 of 500 mg formulation/kg b.w. is used for risk assessment as worst-case. The dietary TER acute values for the screening step and tier 1 risk assessment are presented in Table 9.3‑10.

**Table 9.3‑10: Screening step and tier 1 calculations of the acute risk for mammals due to the use of BAS 736 00 F in “cereals”** **– exposure to formulation with LD50 > 500 mg/kg b.w.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Data from Data\_Entry worksheet** | **Crop** | | | **Application rate (kg formulation/ha)** | **Number of applications** | **Application Interval** | | **DT50** | | **LD50** |  | |
| Cereals | | | 2.156 | 2 | 21 | | 10.0 | | > 500 |  | |
| **Screening step:** | | | | | | | | | | | | |
| Acute risk assessment screening step | **Indicator species** | | | **Shortcut value** | **Daily Dietary Dose (single)** | **MAF (90)** | | **Daily Dietary Dose (Multiple)** | | **TER** | Please perform first tier risk assessment | |
| Small herbivorous mammal | | | 118.4 | 255.27 | 1.1 | | 280.80 | | **> 1.8** |
| **First Tier Risk Assessment:** | | | | | | | | | | | | |
| Calculate TER for each generic focal species | | **Crop** | **Generic focal species** | | | | **Short cut value** | | **TER** | | | No refinement  required |
| Cereals BBCH ≥ 20 | Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods | | | | 5.4 | | > 39.0 | | |
| Cereals BBCH 30 - 39 | Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods | | | | 8.6 | | > 24.5 | | |
| Cereals BBCH ≥ 40 | Small herbivorous mammal "vole Grass + cereals 100% grass | | | | 40.9 | | **> 5.2** | | | **Higher tier risk assessment required** |
| Cereals BBCH ≥ 40 | Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods | | | | 5.2 | | > 40.5 | | | No refinement  required |

TER values in **bold** are below the trigger.

Based on the LD50 > 500 mg/kg b.w. the tier 1 risk assessment resulted in TER values above the trigger of 10 for acceptability of effects for all scenarios, except for the small herbivorous mammal “vole” scenario at BBCH ≥ 40 and a higher tier risk assessment is presented in chapter 9.3.2.2.

**Combined reproductive toxicity**

As requested in the summary report of the Steering Committee of the Central Zone Harmonisation workshop in April 2015 and update of October 2016 (Central Zone Harmonisation Workshop, 2016), a long-term combination toxicity tier 1 risk assessment is presented. As proposed there, the calculations follow the concentration addition model.

The combined TERLT value is calculated according to the following formula:

TERLT combi = trigger/((trigger/TERLT substance 1)+(trigger/TERLT substance 2))

An acceptable risk is expected when TERLT combi > trigger.

The TERLT combi values are calculated based on screening step and tier 1 TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in Table 9.3‑11.

**Table 9.3‑11: Combined reproductive toxicity risk assessment for mammals due to the use of BAS 736 00 F for the crop group “cereals”**

| **Crop scenario and/or indicator species** | | **TERLT 1)**  **fluxapyroxad** | **TERLT 1)**  **azoxystrobin** | **TERLT combi** | **Trigger** |
| --- | --- | --- | --- | --- | --- |
| **Reproductive (screening step)** | | | | | |
| Cereals | Small herbivorous mammal | **3.3** | 6.9 | **2.2** | 5 |
| **Reproductive (tier 1) 1)** | | | | | |
| Cereals BBCH ≥ 20 | Small insectivorous mammal “shrew” | 82.8 | 176.5 | 56.4 | 5 |
| Cereals BBCH 30 - 39 | Small omnivorous mammal “mouse” | 40.3 | 86.0 | 27.4 | 5 |
| Cereals BBCH ≥ 40 | Small herbivorous mammal “vole” | 7.2 | 15.5 | **4.9** | 5 |
| Cereals BBCH ≥ 40 | Small omnivorous mammal “mouse” | 68.4 | 145.8 | 46.5 | 5 |

TER values shown in **bold** fall below the relevant trigger

1) Reproductive TER values are presented in Table 9.3‑6 and Table 9.3‑7.

The TERLT combi values for the relevant scenarios are all above the trigger value of 5 at tier 1, except the small herbivorous mammal “vole” scenario at BBCH ≥ 40, which is just below the trigger with a TERLT combi = 4.9. Therefore, further consideration of the combined reproductive risk to mammals is required for the use in cereals at BBCH ≥ 40 and a higher tier risk assessment is presented in chapter 9.3.2.2.

#### Higher-tier risk assessment

Based on the calculations presented in chapter 9.3.2.1 (first-tier risk assessment), a higher tier risk assessment for dietary exposure is required for the following scenarios:

* Combined acute risk assessment for the small herbivorous mammal “vole” scenario at BBCH ≥ 40 (formulation approach).
* Combined reproductive risk assessment for the small herbivorous mammal “vole” scenario at BBCH ≥ 40.

**Refinement of the combined acute risk assessment of the formulation approach for the small herbivorous mammal “vole” scenario at BBCH ≥ 40**

Please note that two different refinement approaches are presented. In the first approach the acute higher tier risk assessment for the formulation is based on the refinement of the deposition factor, whereas the second approach is based on the interpolated LD50 value of 1848 mg/kg b.w. for BAS 736 00 F as refinement parameter.

*Approach 1: Relevant deposition factor (DF) for cereals at BBCH ≥40*

Following EFSA guidance for evaluating laboratory and field dissipation studies to obtain DegT50 values (EFSA Journal 2014; 12(5):3662, p. 28, Table 1.5) and Ctgb guidance for risk assessment of plant protection products (Ctgb, 2020, p. 14-15, Table 2, version 2.4, January 2020), a **DF of 0.1** for cereals at BBCH 40-69 will be applied for the small herbivorous mammal scenario at BBCH ≥ 40 in cereals.

The refined acute TER for the formulation considering the refinement of the deposition factor is presented in Table 9.3‑12.

**Table 9.3‑12: BAS 736 00 F: Refined acute dietary risk assessment for the small herbivorous mammal “vole” scenario in cereals based on a refined deposition factor – approach 1**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Food type** | **FIR/bw** | **PDi, fresh** | **PT** | **RUD90 [mg a.s./kg]** | **MAF90** | **fTWA** | **DF** | **Use rate [kg a.s./ha]** | **DDD [mg a.s./kg b.w./d]** |
| **Grasses/**  **Cereal shoots** | 1.334 | 1.0 | 1.0 | 102.3 | 1.14~~)~~ | 1 | 0.1 1) | 2.156 | 33.535 |
| **Toxicity endpoint [mg a.s./kg b.w./d]** | | | | | | | | | > 500 |
| **TERA** | | | | | | | | | > 14.91 |

1) Refined parameter

|  |
| --- |
| **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. Therefore, the Applicant proposal was accepted. |

*Approach 2: Refined acute endpoint from the formulation study*

It is acknowledged that the rat toxicity study that was conducted for classification and labeling purposes is not suitable to derive precise LD50 values. The dose of 500 mg/kg b.w. did not lead to any mortality and thus the use of this dose level as surrogate for an LD50 represents an unrealistic assessment of the risk. Therefore, the interpolated LD50 of 1848 mg/kg b.w. for BAS 736 00 F (see chapter 9.3.1) has been considered in the refined risk assessment.

The refined combined acute TER for the formulation considering the refined acute endpoint is presented in Table 9.3‑13.

**Table 9.3‑13: BAS 736 00 F: Refined acute dietary risk assessment** **for the small herbivorous mammal “vole” scenario in cereals based on the interpolated LD50 value – approach 2**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Food type** | **FIR/bw** | **PDi, fresh** | **PT** | **RUD90 [mg a.s./kg]** | **MAF90** | **fTWA** | **DF** | **Use rate [kg a.s./ha]** | **DDD [mg a.s./kg b.w./d]** |
| **Grasses/**  **Cereal shoots** | 1.334 | 1.0 | 1.0 | 102.3 | 1.14 | 1 | 0.3 | 2.156 | 97.074 |
| **Toxicity endpoint [mg ~~a.s.~~ formulation/kg b.w./d]** | | | | | | | | | 1848/1667 1) |
| **TERA** | | | | | | | | | 18.4/17.2 |

1) Refined parameter

The refined TERA for the formulation toxicity of BAS 736 00 H resulted in TER values above the trigger of 10 for acceptability of effects for the small herbivorous mammal “vole” scenario at BBCH ≥ 40 for both approaches. Therefore, the acute dietary risk to mammals from BAS 736 00 H is acceptable.

|  |
| --- |
| **Review Comments:**  An acute oral toxicity study with BAS 736 00 F in rats was evaluated in mammalian toxicology assessment. For more details about fitted of the dose-response curve via the probit model according to Finney, please refer to section B6. |

**Refinement of the combined reproductive risk assessment for the small herbivorous mammal “vole” scenario at BBCH ≥ 40**

In the summary report of the Steering Committee of the Central Zone Harmonisation workshop in April 2015 and update of October 2016, the Member States did not provide specific guidance for applicants and risk assessors from the competent regulatory authorities on how to proceed in case the tier 1 calculation fails the trigger. In the words of the summary report, “refinement options [for the long-term combined toxicity risk assessment] remain unclear.” To address the issue in this case, the applicant has decided to refine the tier 1 risk assessment for each active ingredient and use these refined TER values in a higher tier combined reproductive toxicity risk assessment.

The reproductive higher tier risk assessment is based on the refinement of the deposition factor as introduced in the acute higher tier risk assessment above.

The refined long-term TERis provided in Table 9.3‑14 for fluxapyroxad and Table 9.3‑15 for azoxystrobin. Using these refined TERs for the active substances, the TERLT combi are provided in Table 9.3‑16.

**Table 9.3‑14: Fluxapyroxad: Refined reproductive dietary risk assessment for the “vole” (common vole) scenario in cereals at BBCH ≥ 40**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Food type** | **FIR/bw** | **PDi, fresh** | **PT** | **RUD [mg a.s./kg]** | **MAFm** | **fTWA** | **DF** | **Use rate [kg a.s./ha]** | **DDD [mg a.s./kg b.w./d]** |
| **Grasses/**  **Cereal shoots** | 1.334 | 1.0 | 1.0 | 54.2 | 1.23 | 0.53 | 0.1 1) | 0.10 | 0.471 |
| **Toxicity endpoint [mg a.s./kg b.w./d]** | | | | | | | | | 10 |
| **TERLT** | | | | | | | | | 21.2 |

1) Refined parameter

**Table 9.3‑15: Azoxystrobin: Refined reproductive dietary risk assessment for the “vole” (common vole) scenario in cereals at BBCH ≥ 40**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Food type** | **FIR/bw** | **PDi, fresh** | **PT** | **RUD [mg a.s./kg]** | **MAFm** | **fTWA** | **DF** | **Use rate [kg a.s./ha]** | **DDD [mg a.s./kg b.w./d]** |
| **Grasses/**  **Cereal shoots** | 1.334 | 1.0 | 1.0 | 54.2 | 1.23 | 0.53 | 0.1 1) | 0.15 | 0.707 |
| **Toxicity endpoint [mg a.s./kg b.w./d]** | | | | | | | | | 32 |
| **TERLT** | | | | | | | | | 45.3 |

1) Refined parameter

**Table 9.3‑16: Cereals: Refined combination toxicity risk assessment for the “vole” scenario in cereals at BBCH ≥ 40**

| **Crop scenario and/or indicator species** | | **TERLT (higher tier)**  **fluxapyroxad** | **TERLT (higher tier)**  **azoxystrobin** | **TERLT combi** | **Trigger** |
| --- | --- | --- | --- | --- | --- |
| **Reproductive – Higher tier** | | | | | |
| Cereals BBCH ≥ 40 | Small herbivorous mammal "vole” | 21.2 | 45.3 | 14.4 | 5 |

|  |
| --- |
| **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. Therefore, the Applicant proposal was accepted. |

**Summary and conclusions of the combined acute and long-term dietary risk assessments**

The acute risk assessment for the combined toxicity of the active substances resulted in TERA values above the trigger of 10 for acceptability of effects both for the virtual compound and formulation approaches, with exception of the small herbivorous mammal “vole” scenario at BBCH ≥ 40. Based on the refinement of the deposition factor (approach 1) and the interpolated LD50 value (approach 2) an acceptable acute risk to small herbivorous mammal could be identified in both approaches.

The TERLT combi values at tier 1 risk assessment are all above the trigger value of 5 for the relevant scenarios, except small herbivorous mammal “vole” scenario at BBCH ≥ 40. Based on the higher tier risk assessment considering the refinement of the deposition factor for both active substances an acceptable combined long-term risk to small herbivorous mammal could be identified.

In conclusion, the risk assessment shows that combined dietary acute and reproductive risks to mammals from the intended use of BAS 736 00 F are 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 b.w./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).

The ratio calculations for effective application rate to relevant endpoint are detailed in Table 9.3‑17 and Table 9.3‑18. The ratios for acute and reproductive endpoints for fluxapyroxad (< 0.1 and 19.1, respectively) and for azoxystrobin (< 0.05 and 8.6, respectively) do not exceed the threshold values of 3000 and 50, respectively, thus no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary. Therefore, a quantitative drinking water risk assessment for the puddle scenario is not triggered.

**Table 9.3‑17: Assessment of the risk for mammals due to exposure to fluxapyroxad via contaminated drinking water in puddles**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Fluxapyroxad** | **Reference** |
| Kfoc (geometric mean) [L/kg] | 681 | Chapter 8.5.1 |
| DT50 (soil) (geometric mean) [days] | 151 | Chapter 8.9 |
| Number of applications | 2 | Chapter 9.1 |
| Interval [days] | 21 | Chapter 9.1 |
| MAFm 1) | 1.91 | -- |
| Max use rate [g/ha] | 100 | Chapter 9.1 |
| AReff [g/ha] 2) | 191 | -- |
| LD50 [mg/kg b.w.] | > 2000 | Chapter 9.3.1 |
| Ratio (acute) 3) | < 0.1 | -- |
| NO(A)EL [mg/kg b.w./d] | 10 | Chapter 9.3.1 |
| Ratio (repro) 3) | 19.1 | -- |
| 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 MAFm

3) Ratio of AReff and relevant toxicity endpoint

**Table 9.3‑18: Assessment of the risk for mammals due to exposure to azoxystrobin via contaminated drinking water in puddles**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Azoxystrobin** | **Reference** |
| Koc (geometric mean) [L/kg] | 392 | Chapter 8.5.1 |
| DT50 (soil) (geometric mean) [days] | 78 | Chapter 8.9 |
| Number of applications | 2 | Chapter 9.1 |
| Interval [days] | 21 | Chapter 9.1 |
| MAFm 1) | 1.83 | -- |
| Max use rate [g/ha] | 150 | Chapter 9.1 |
| AReff [g/ha] 2) | 274.5 | -- |
| LD50 [mg/kg b.w.] | > 5000 | Chapter 9.3.1 |
| Ratio (acute) 3) | < 0.05 | -- |
| NO(A)EL [mg/kg b.w./d] | 32 | Chapter 9.3.1 |
| Ratio (repro) 3) | 8.6 | -- |
| Trigger | 50 | -- |
| 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 MAFm

3) Ratio of AReff and relevant toxicity endpoint

In conclusion, the risk to mammals via drinking water from the intended use of BAS 736 00 F according to the proposed use pattern is acceptable.

#### Effects of secondary poisoning

The log Pow of the active substance fluxapyroxad is 3.1 (BASF DocID 2007/1057001), which triggers an assessment of the potential risk from secondary poisoning.

The log Pow of azoxystrobin is 2.5 (EFSA Journal 2010; 8(4):1542) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

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.

As shown in the following Table 9.3‑19, the TERLT for fluxapyroxad exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating mammals via secondary poisoning.

**Table 9.3‑19: Assessment of the risk for earthworm-eating mammals due to exposure to fluxapyroxad via bioaccumulation in earthworms (secondary poisoning) for the intended use**

| **Parameter** | **Fluxapyroxad** | **Reference** |
| --- | --- | --- |
| PECsoil (accu) [mg/kg soil] 1) | 0.078 | Chapter 8.7 |
| Kow | 1259 | BASF DocID 2007/1057001 |
| Koc (geometric mean) [L/kg] | 681 | Chapter 8.5.1 |
| foc (default) | 0.02 | EFSA/2009/1438 |
| BCF 2) | 1.171 | -- |
| PECworm [mg/kg] 3) | 0.091 | -- |
| Daily dose [mg/kg b.w./d] | 0.117 | -- |
| NO(A)EL [mg/kg b.w./d] | 10 | Chapter 9.3.1 |
| TERlt | 85.5 | -- |

1) Worst case PECsoil (accu) value was calculated for an application scenario of 2 x 100 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

2) Bioconcentration factor (BCF) = (0.84 + 0.012 x Kow) / (foc x Koc)

3) PECworm = PECsoil x BCF

4) Daily dose = 1.28 x PECworm

5) TERLT = NO(A)EL / Daily dose.

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 9.3‑20, the TERLT for both fluxapyroxad exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating mammals via secondary poisoning.

**Table 9.3‑20: Assessment of the risk for fish-eating mammals due to exposure to fluxapyroxad via bioaccumulation in fish (secondary poisoning) for the intended use**

| **Parameter** | **Fluxapyroxad** | **Reference** |
| --- | --- | --- |
| PECsw (twa, 21 d) [mg/L] 1) | 6.123 × 10-3 | Chapter 8.9 (Step 2, Northern Europe) |
| BCFfish (max. worst case) | 36 | EFSA Journal 2012;10(1): 2522 |
| PECfish [mg/kg] 2) | 0.220 | -- |
| Daily dose [mg/kg b.w./d] 3) | 0.031 | -- |
| NO(A)EL [mg/kg b.w./d] | 10.0 | Chapter 9.3.1 |
| TERlt 4) | 319.5 | -- |

1) PECsw (twa, 21 d) value calculated for a multiple application scenario of 2 x 100 g a.s./ha to cereals from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

2) PECfish = PECsw (twa, 21 d) x BCF

3) Daily dose = 0.142 x PECfish

4) TERLT = NO(A)EL / Daily dose.

#### Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU review of fluxapyroxad (EFSA Journal 2012; 10(1): 2522).

No evidence of accumulation in animal tissue was concluded in the EU review of azoxystrobin from toxicokinetics studies (EFSA Journal 2010; 8(4): 1542).

Since the bioaccumulation potential of both fluxapyroxad and azoxystrobin is low no further assessment on biomagnification 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 736 00 F according to good agricultural practice is acceptable.**

|  |
| --- |
| **Review Comments:**  The acute and chronic risks ofBAS 736 00 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 736 00 F in rats was taken to consideration in the evaluation.  All TER values exceed the relevant triggers in the screening step or Tier 1 risk assessment for fluxapyroxad, azoxystrobin and for combined active substances (virtual compound approach). Based on the higher tier risk assessment, where the deposition factor was modified, for the formulation (acute) and for combined active substances risk assessment (chronic), the TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects. Therefore, BAS 736 00 F does not pose an unacceptable risk to mammals following applications according to recommended use pattern.  Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is low. |

**References**

Ctgb. 2020. Evaluation Manual for the Authorisation of plant protection products according to Regulation (EC) No 1107/2009. EU part. Plant protection products. Chapter 7 Ecotoxicology: terrestrial; birds and mammals. version 2.4; January 2020. Board for the Authorisation of plant protection products and biocides (ctgb).

EFSA/2014/3662. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014; 12(5):3662.

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

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to terrestrial life-stages of amphibians and reptiles shall be addressed, yet toxicity testing is not required.

In general, information on the toxicity of chemicals to terrestrial life-stages of amphibians is scarce. However, in the cases where terrestrial life-stages of amphibians were tested in the same type of study as birds and mammals, the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 12 and 13 in Fryday and Thompson, 2012). A review compiling data on 26 chemicals for birds, mammals and amphibians confirmed this pattern (Crane et al., 2016).

For reptiles, there is even less information available than for amphibians (see the review by Fryday and Thompson, 2009).

For the time being, it is assumed that the risk assessments for birds and mammals are protective for terrestrial life-stages of amphibians and reptiles; an approach that is also used by US-EPA (US-EPA 2004).

**References**

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Crane, M., Finnegan, M., Weltje, L., Kosmala-Grzechnik, S., Gross, M. and Wheeler, J.R. 2016. Acute oral toxicity of chemicals in terrestrial life stages of amphibians: Comparisons to birds and mammals. Regulatory Toxicology and Pharmacology, 80: 335-341.

Fryday, S. and Thompson, H. 2009. Compared toxicity of chemicals to reptiles and other vertebrates. EFSA Supporting Publications, 6, EN-14: 169 pp.

Fryday, S. and Thompson, H. 2012. Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural environments. EFSA Supporting Publications, 9, EN-343: 348 pp.

US-EPA 2004. Overview of the ecological risk assessment process in the Office of Pesticide Programs, U.S. Environmental Protection Agency: Endangered and Threatened Species Effects Determinations. Office of Prevention, Pesticides and Toxic Substances; Office of Pesticide Programs, Washington, D.C. 92 pp.

## Effects on aquatic organisms (KCP 10.2)

### Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with the formulation BAS 736 00 F, the active substances fluxapyroxad (BAS 700 F), azoxystrobin (BAS 9164 F) and their relevant metabolites. Full details of these studies are provided in the EU DAR of fluxapyroxad (DAR, Vol. 1, B.9, Feb. 2011) and azoxystrobin (DAR Vol. 1 B.9 May 20079 and EFSA conclusions of fluxapyroxad (EFSA Journal 2012; 10(1):2522), azoxystrobin (EFSA Journal 2010; 8(4):1542) and related documents, as well as in Appendix 2 of this document (new studies).

The selection of studies and endpoints and the risk assessments (RAs) for fluxapyroxad and azoxystrobin are generally in line with the results of the EU review process (justification in case of deviations is provided below).

Effects on aquatic organisms of product BAS 736 00 F were not evaluated previously as part of the EU assessment of the active substances. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Appropriate RAs for aquatic organisms for the active substances, its major metabolites and the formulated product BAS 736 00 F for the proposed use pattern are provided based on available toxicity data.

Full references to cited literature are given at the end of this document.

**Fluxapyroxad and metabolites**

The results from toxicity tests with representative aquatic species conducted with the active substance fluxapyroxad and its metabolites found in aquatic systems are summarized in Table 9.5‑1.

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

| **Species** | **Substance** | **Exposure**  **System** | **Results** | **Reference / BASF DocID** |
| --- | --- | --- | --- | --- |
| *Oncorhynchus mykiss* | fluxapyroxad | 96 h, s | LC50 = 0.546 mg a.s./L nom | EFSA Journal 2012; 10(1):2522 2007/1057974 |
| *Lepomis macrochirus* | fluxapyroxad | 96 h, s | LC50 =1.15 mg a.s./L mm | EFSA Journal 2012; 10(1):2522 2008/1010605 |
| *Pimephales promelas* | fluxapyroxad | 96 h, s | LC50 =0.466 mg a.s./L mm | EFSA Journal 2012; 10(1):2522 2009/1025414 |
| *Cyprinus carpio* | fluxapyroxad | 96 h, ss | **LC50 = 0.290 mg a.s./L mm** | EFSA Journal 2012; 10(1):2522 2008/1064998 |
| *Cyprinodon variegatus 1)* | fluxapyroxad | 96 h, s | LC50 = 1.30 mg a.s./L mm | EFSA Journal 2012; 10(1):2522 2008/7015417 |
| Fish (acute) SSD  calculations  (96 h NOEC data for 5 fish species) | fluxapyroxad | -- | HC5 fish, acute = 0.148 mg a.s./L | New calculations  (see below) |
| *Pimephales promelas* | fluxapyroxad | 33 d (ELS), f | **NOEC = 0.0359 mg a.s./L mm** | EFSA Journal 2012; 10(1):2522 2008/1090791  (+Amendment:  2016/1027732) |
| **~~NOEC = 0.0676 mg a.s./L~~~~mm~~~~\*~~** | ~~New re-evaluation~~  ~~2020/2095131~~ |
| *Lepomis macrochirus* | fluxapyroxad | Bioconcentration study with 28 d exposure, 16 d depuration) | BCF (whole fish) = 37 | EFSA Journal 2012; 10(1):2522 2009/1012801 |
| *~~Daphnia magna~~* | ~~fluxapyroxad~~ | ~~48 h, s~~ | **~~EC~~~~50~~ ~~= 6.78 mg a.s./L~~~~mm~~** | ~~EFSA Journal 2012; 10(1):2522 2008/1028252~~ |
| *~~Americamysis bahia~~~~1)~~* | ~~fluxapyroxad~~ | ~~48 h, s~~ | **~~LC~~~~50~~ ~~= 6.1 mg a.s./L~~~~mm~~~~2)~~** | ~~EFSA Journal 2012; 10(1):2522 2009/7000069~~ |
| *Crassostrea virginica 1)* | fluxapyroxad | 48 h, f | **EC50 ~~>~~ = ~~2.2~~ 1.1 mg a.s./L mm2)** | EFSA Journal 2012; 10(1):2522 2009/7000165 |
| *Daphnia magna* | fluxapyroxad | 21 d, s | **NOEC = 0.500 mg a.s./L nom** | EFSA Journal 2012; 10(1):2522 2008/1055084 |
| *Chironomus riparius* | fluxapyroxad | 28 d, spiked sediment | **NOEC = 75.9 mg a.s./kg sed. (dw) im** | EFSA Journal 2012; 10(1):2522 2009/1037085 |
| *Pseudokirchneriella subcapitata  (Syn. Ankistrodesmus bibraianus)* | fluxapyroxad | 72 h, s | **ErC50 = 0.700 mg a.s./L nom 3)** EyC50 =0.400 mg a.s./L nom | EFSA Journal 2012; 10(1):2522 2008/1022788  (+ Amendment: 2009/1015272, 2010/1016358) |
| *Anabaena flos-aquae* | fluxapyroxad | 72 h, s | ErC50 = 2.61 mg a.s./L mm 3) EyC50 =1.38 mg a.s./L mm | EFSA Journal 2012; 10(1):2522 2009/1079883 |
| *Navicula pelliculosa* | fluxapyroxad | 72 h, s | ErC50 > 3.42 mg a.s./L mm 3) EyC50 = 2.31 mg a.s./L mm | EFSA Journal 2012; 10(1):2522 2009/1079885 |
| *Lemna gibba* | fluxapyroxad | 7 d, s | **ErC50 > 3.43 mg a.s./L mm 3)**EyC50 = 2.19 mg a.s./L mm | EFSA Journal 2012; 10(1):2522 2009/1086122 |
| *Oncorhynchus mykiss* | M700F001 | 96 h, s | LC50 > 100 mg/L nom | EFSA Journal 2012; 10(1):2522 2009/1021591 |
| *Oncorhynchus mykiss* | M700F002 | 96 h, s | LC50 > 100 mg/L nom | EFSA Journal 2012; 10(1):25222009/1021595 |
| *Oncorhynchus mykiss* | M700F007 | 96 h, s | LC50 > 100 mg/L nom | EFSA Journal 2012; 10(1):2522 2009/1026001 |
| *Daphnia magna* | M700F001 | 48 h, s | EC50 > 100 mg/L nom | EFSA Journal 2012; 10(1):2522 2009/1021592 |
| *Daphnia magna* | M700F002 | 48 h, s | EC50 > 100 mg/L nom | EFSA Journal 2012; 10(1):2522 2009/1021596 |
| *Daphnia magna* | M700F007 | 48 h, s | EC50 > 100 mg/L nom | EFSA Journal 2012; 10(1):2522 2009/1026002 |
| *Pseudokirchneriella subcapitata* | M700F001 | 72 h, s | **ErC50 =36.31 mg/L nom 3)**EyC50 = 26.42 mg/L nom | EFSA Journal 2012; 10(1):2522 2009/1021593  (+ Amendment 2009/1102103) |
| *Pseudokirchneriella subcapitata* | M700F002 | 72 h, s | **ErC50 =26.52 mg/L nom 3)**EyC50 = 22.44 mg/L nom | EFSA Journal 2012; 10(1):2522 2009/1021597 |
| *Pseudokirchneriella subcapitata* | M700F007 | 72 h, s | **ErC50** / EyC50 **> 100 mg/L nom** | EFSA Journal 2012; 10(1):2522  2009/1026003  (+Amendment 2009/1102104)  (+ new recalculation:  2020/2033571) |

**Bold** figures: Endpoint used in standard tier 1 risk assessment if more than one endpoint is available for the respective group or organism.

Abbreviations: ELS = early life stage, BCF = bioconcentration factor, s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations; dw: dry weight

+ In accordance with the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 96-h alga study are considered as relevant endpoints and are presented here.

\* Chronic fish endpoint is diverging from EFSA conclusion since has been recalculated due to new statistical requirements stated in the relevant OECD test guideline 210 (OECD 2013). Therefore, the new endpoint will be used in the risk assessment.

1) Marine species

2) According to the new regulation 283/2013, the 48-h endpoint obtained in the 96-h study is considered as relevant for the risk assessment.

3) In accordance with the EFSA Aquatic Guidance Document (EFSA, 2013) only the EC50 values determined for the endpoint 'growth rate' (ErC50) are considered for the risk assessment for aquatic primary producers if both “growth rate” and “yield / biomass” endpoints are available.

**Azoxystrobin and metabolites**

The results from toxicity tests with representative aquatic species conducted with the active substance azoxystrobin and its metabolites found in aquatic systems are summarized in Table 9.5‑2.

Table 9.5‑2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – azoxystrobin and relevant metabolites

| Species | Substance | Exposure System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Oncorhynchus mykiss* | azoxystrobin | 96 h, f | **LC50 = 0.47 mg a.s./L mm** | EFSA Journal 2010; 8(4):1542,  Cornish, S., Caunter, J.,Sankey S.,Craig, N., 1993, ICI5504/0909 |
| *Lepomis macrochirus* | azoxystrobin | 96 h, f | LC50 = 1.1 mg a.s./L mm | EFSA Journal 2010; 8(4):1542,  Cornish, S., Maddock, B., Kent, S., Sankey S., 1993, ICI5504/0910 |
| *Pimephales promelas* | azoxystrobin | 33 d (ELS), f | NOEC = 0.147 mg a.s./L mm | EFSA Journal 2010; 8(4):1542,  Rhodes, J.E., 1994, ICI5504/0924 |
| *Daphnia magna* | azoxystrobin | 48 h, s | **EC50 = 0.23 mg a.s./L mm** | EFSA Journal 2010; 8(4):1542,  Farrelly, E., Hamer, M.J., 1994, ICIA5504/0931 |
| *Macrocyclops fuscus* | azoxystrobin | 48 h, s | EC50 = 0.13 mg a.s./L nom | EFSA Journal 2010; 8(4):1542,  Farrelly, E., Kearson, L.L., Rapley, J.H., Hamer, 1995, ICIA5504/0940 |
| *Mysidopsis bahia* | azoxystrobin | 96 h, s | EC50 = 0.055 mg a.s./L nom | EFSA Journal 2010; 8(4):1542,  Kent, S.J., Sankey, S.A., Grinell, A.J., 1993, ICI5504/0925 |
| *Mysidopsis bahia* | azoxystrobin | 48 h, s | EC50 = 0.068 mg a.s./L nom | EFSA Journal 2010; 8(4):1542  Ward TJ, Magazu JP, Boeri RL, ICI5504/0958 |
| *Crassostrea gigas* | azoxystrobin | 48 h, s | EC50 = 1.3 mg a.s./L nom | EFSA Journal 2010; 8(4):1542,  Kent, S.J., Sankey, S.A., Cautner, J.E., Grinell, A.J., 1994, ICI5504/0927 |
| *Daphnia magna* | azoxystrobin | 21 d, s | **NOEC = 0.044 mg a.s./L mm** | EFSA Journal 2010; 8(4):1542,  Rapley et al., 1994, ICI5504/0957 |
| *Mysidopsis bahia* | azoxystrobin | 21 d, s | NOEC = 0.00954 mg a.s./L mm | EFSA Journal 2010; 8(4):1542,  Boeri, R.L., Magazu J.P., Ward, T.J., 1997, ICI5504/0952 |
| *Chironomus riparius*  (spiked water) | azoxystrobin | 28 d, s | NOEC = 0.8 mg a.s./L nom | EFSA Journal 2010; 8(4):1542,  Rapley, J.H. & Gentle, W.E., 1997, ICI5504/0956 |
| *Chironomus riparius*  (spiked sediment) | azoxystrobin | 28 d, s | NOEC = 23 mg a.s./kg nom | Azoxystrobin DAR 2010,  Gentle, W.E., 1997, ICI5504/0954 |
| *Pseudokirchneriella subcapitata* | azoxystrobin | 72 h, s | EC50 = 0.183 mg a.s./L nom1)  EC50 = 0.36 mg a.s./L (m) | EFSA Journal 2010; 8(4):1542,  Grinell A., Sankey S., Craig N., Kent S., Smyth D.1993, ICI5504/0961 |
| *Skeletonema costatum* | azoxystrobin | 72 h, s | EbC50 = 0.098 mg a.s./L nom  ErC50 = 0.3 mg a.s./L (n) | EFSA Journal 2010; 8(4):1542,  Smyth, D.V., Kent, S.J., Sankey, S.A., Johnson, P.A., 1994, ICI5504/0966 |
| *Navicula pelliculosa* | azoxystrobin | 120 h, s | EbC50 = 0.014 mg a.s./L nom  **ErC50 = 0.146** mg a.s./L (n) | EFSA Journal 2010; 8(4):1542,  Smyth, D.V., Sankey, S.A., Kent, S.J., Stanley, R.D., 1994, ICI5504/0965 |
| *Anabaena flos-aquae* | azoxystrobin | 120 h, s | EbC50 = 9.5 mg a.s./L nom  ErC50 = 13.9 mg a.s./L (m) | EFSA Journal 2010; 8(4):1542,  Smyth, D.V., Kent, S.J., Sankey, S.A., Shearing, J.M., 1994, ICI5504/0967 |
| ~~Geomean (EC/E~~~~b~~~~C~~~~50~~ ~~data for 3 algal species)~~~~2)~~ | ~~azoxystrobin~~ | ~~--~~ | **~~Geomean-EC~~~~50~~ ~~= 0.063 mg a.s./L~~** | ~~--~~ |
| *Lemna gibba* | azoxystrobin | 14 d, ss | EC50 = 3.2 mg a.s./L nom | EFSA Journal 2010; 8(4):1542,  Smyth, D.V., Sankey, S.A., Kent, S.J., Stanley, R.D., 1994a, ICI5504/0963 |
| *Oncorhynchus mykiss* | R234886 | 96 h, f | LC50 > 150 mg/L mm | EFSA Journal 2010; 8(4):1542,  Kent et al, 1993, ICI15504/0913 |
| *Oncorhynchus mykiss* | R402173 | 96 h, s | LC50 = 62 mg/L mm | EFSA Journal 2010; 8(4):1542,  Wallace, S.J., 2002, SYN511114/0001 |
| *Oncorhynchus mykiss* | R401553 | 96 h, s | LC50 > 120 mg/L nom | EFSA Journal 2010; 8(4):1542,  Bowles, A.J., Wallace, S.J., 2002, SYN501657/0002 |
| *Daphnia magna* | R234886 | 48 h, s | EC50 > 180 mg/L nom | EFSA Journal 2010; 8(4):1542,  Johnson P., Banner A., Sankey S., Kent S., 1993, ICI5504/0926 |
| *Daphnia magna* | R402173 | 48 h, s | EC50 > 100 mg/L nom | EFSA Journal 2010; 8(4):1542,  Wallace, S.J., 2002a, SYN501114/0002 |
| *Daphnia magna* | R401553 | 48 h, s | EC50 > 120 mg/L nom | EFSA Journal 2010; 8(4):1542,  Bowles, A.J., Wallace, S.J., 2002a, SYN501657/0003 |
| *Pseudokirchneriella subcapitata* | R234886 | 72 h, s | EbC50 = 47 mg/L mm | EFSA Journal 2010; 8(4):1542,  Smyth et al, 1993, ICI5504/0962 |
| *Pseudokirchneriella subcapitata* | R402173 | 72 h, s | EbC50 = 67 mg/L nom  ErC50 = 67 mg a.s./L (n) | EFSA Journal 2010; 8(4):1542,  Wallace, S.J., Woodyer, J.M., 2002, SYN511114/0003 |
| *Pseudokirchneriella subcapitata* | R401553 | 72 h, s | EbC50 > 120 mg/L nom  ErC50 > 120 mg a.s./L (n) | EFSA Journal 2010; 8(4):1542,  Bowles, A.J., Wallace, S.J., 2002b, SYN501657/0004 |
| **Higher-tier studies (micro- or mesocosm studies)** | | | | |
| The mesocosm study is considered to be a well-conducted mesocosm with an appropriate diversity and abundance of species. It should be noted that azoxystrobin was only applied once, and concentrations were only measured 21 hours after application and not throughout the course of the study. Species/groups were present in sufficient numbers to allow appropriate statistical analysis.  The Notifier proposed that the no observed ecologically adverse effects concentration (NOEAEC) is 10 μg/L. No uncertainty or assessment factor was proposed.  From the summary above it can be concluded that there were effects at all concentrations, hence it is not possible to establish a NOEC. The treatment related effects at 10 μg/L were considered to be relatively short-lived and restricted to decreases in the following parameters:   * Daphnia spp. – effects at 10 μg/L were noted at 3, 7 and 14 days * Total cladocera – effects at 10 μg/L were noted at 3, 7 and 14 days * Copepoda nauplii – effects at day 35 * Copepoda Cyclopoid copepodites – effects at 10 μg/L were noted at days 7 and 10, * Copepoda Cyclopoid adults – effects were noted on day 3 only * Sphaeriidae – significantly fewer on days 72 and 93 for samples collected via nets, there were significantly fewer on days 22, 30 44 and 72. * Total mollusc – in samples collected via nets were lower on days 22 and 72 * Total macroinvertebrates – in sample collected via nets were lower on day 30.   The following groups increased and were probably the result of indirect effects:   * Chydorus – significantly greater numbers on study day 10 and 28 * Pompholyx sp – significantly greater numbers than the control on day 14 only * Testudinella sp – there were significantly greater numbers than the control on days 42 and 35. * Total rotifer – there were significantly greater numbers than the control on days 3, 35, 42 and 56.   It should however be noted that there was only one application and there was only chemical analysis 21 hours after application; due to this it is proposed that the effect concentrations should be based on the initial nominal concentrations.  **The acute and chronic RAC for aquatic invertebrates should be set at 3.3 μg a.s./L** | | | | EFSA Journal 2010; 8(4):1542,  Cole, J.F.H., Everett, C.J., Gentle, W., Ashwell, J.A., Goggin, U., 2000, ICI5504/0976 |

Abbreviations: s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

**Bold** figures: Endpoint used in standard tier 1 risk assessment if more than one endpoint is available for the respective group or organism.

1) The 72- and 96-hour EC50 values of azoxystrobin for *Pseudokirchneriella subcapitata* (formerly known as Selenastrum capricornutum) are 0.183 mg/L and 0.36 mg/L respectively; it is in the belief of Syngenta that the 72-h EC50 endpoint is erroneously presented as 0.36 mg/L in the EFSA Conclusion (2010); 8(4):1542.

2) Proposed new acute endpoint for algaefor azoxystrobin. Geomean of *Pseudokirchneriella subcapitata* (0.183 mg a.s./L nom), *Skeletonema costatum* (0.098 mg a.s./L nom) and *Navicula pelliculosa* (0.014 mg a.s./L nom). For details, please refer to chapter 9.5.1.1

**Formulated product (BAS 736 00 F)**

The results from the toxicity tests with representative aquatic species conducted with the formulation BAS 736 00 F found in aquatic systems are summarized in Table 9.5‑3.

Table 9.5‑3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – BAS 736 00 F

| Species | Substance | Exposure  System | Results | Reference |
| --- | --- | --- | --- | --- |
| *Oncorhynchus mykiss* | BAS 736 00 F | 96 h, s | LC50 = 2.83 mg/L nom | New Study – Not EU agreed  2019/2039621 |
| *Daphnia magna* | BAS 736 00 F | 48 h, s | EC50 = 1.57 mg/L nom | New Study – Not EU agreed  2019/2039622 |
| *Pseudokirchneriella subcapitata* | BAS 736 00 F | 72 h, s | ErC50 = 4.19 mg/L nom  EyC50 = 1.46 mg/L nom | New Study – Not EU agreed  2019/2039623 |

Abbreviations: s: static; nom: based on nominal concentrations

#### Justification for new endpoints

**Fluxapyroxad**

For fluxapyroxad, an acute study on the marine crustacean species *A. bahia* was submitted for Annex I inclusion*.* In the LoEP (list of endpoints) in the EFSA Peer review Report for fluxapyroxad (2012), the 96-h LC50 value(*i.e.* 3.6 mg a.s./L) resulting from this study is listed. This EU agreed endpoint is used for the risk assessment. The higher 48-h LC50 value (*i.e.* 6.1 mg a.s./L) is not considered for the risk assessment.

Additionally, diverging from the EFSA conclusion for fluxapyroxad and according to new statistical requirements according to OECD TG 210 (OECD 2013), the endpoint for the chronic fish study on *Pimephales promelas* (i.e., NOEC = 0.0359 mg a.s./L) has been recalculated to NOEC = 0.0676 mg a.s./L. The recalculated value will be considered in the risk assessment.

In addition, a Species Sensitivity Distribution (SSD) has been calculated based on the no observed effect concentration (NOEC) endpoints obtained from acute studies using 5 different fish species (i.e. *O. mykiss*, *L. macrochirus*, *P. promelas*, *C. carpio* and *C. variegatus*). The resulting HC5 is used to refine the RA of the active substance fluxapyroxad. Please refer to refined risk assessment.

|  |
| --- |
| **Review Comments:**  The zRMS is of the opinion that the endpoints given in the LoEP should be considered for the risk assessment. Therefore, the endpoint of 1.1 mg a.s./L for *C. virginica* will be considered for the acute risk assessment for aquatic invertebrates.  In addition, a refined risk assessment for fish based on SSD calculation is not required, as safe use can be concluded using FOCUS Step 3 values. |

**Azoxystrobin**

Acute and chronic aquatic invertebrate endpoint for azoxystrobin

In the case of azoxystrobin, laboratory acute toxicity data are available for 12 freshwater and two marine invertebrate species. The effects of azoxystrobin on phytoplankton, zooplankton and macro-invertebrate populations have also been evaluated in an outdoor mesocosm study. The NOAEC from this mesocosm study was considered to be 10 µg a.s./L.

These studies were evaluated during the Annex 1 Review and were used to derive a regulatory acceptable concentration (RAC) for azoxystrobin. Several approaches were considered in the derivation of a RAC.

* Geometric mean of acute laboratory endpoints of 8.9 µg a.s./L
* Lower limit of the HC5 based on acute laboratory data of 7.15 µg a.s./L
* Mesocosm study NOAEC of 10 µg a.s./L.

Following consideration of all lines of evidence, the RAC for azoxystrobin was considered to be equivalent to the mesocosm NOAEC of 10 µg a.s./L with a safety factor of 3, i.e. 3.3 µg a.s./L and was solely used for the risk assessment.

|  |
| --- |
| **Review Comments:**  The RAC value of 3.3 µg/L was accepted by zRMS to refine acute and chronic risk to aquatic invertebrate. |

Algal endpoint for azoxystrobin

The assessment will be refined by consideration of all available algal data for azoxystrobin. In addition to the data for the standard freshwater species, *Pseudokirchneriella subcapitata (previously known as Selenastrum capricornutum),* data are also available for the freshwater species, *Navicula pelliculosa* and *Anabaena flos-aquae* species, and the marine species *Skeletonema costatum*. Recently the EFSA Aquatic GD (2013) indicates that in cases where legislation requires the testing of a single standard species but data are available for more than one species, risk assessments may be performed using the geometric mean of available endpoints and the standard safety factor.

The EFSA conclusion for azoxystrobin uses a refined algal endpoint of 262 µg a.s./L, based on endpoints of 360, 98, 14 and 9500 µg a.s./L. However, the value of 360 µg a.s./L quoted for *Pseudokirchneriella subcapitata* represents the 96-h endpoint whereas EU risk assessments are typically based on 72-h endpoints. Consequently, the correct geometric mean of 72-h EbC50 values for the available species is 221 µg a.s./L based on endpoints of 183, 98, 14 and 9500 µg a.s./L. However, the endpoint for *Anabaena flos-aquae* of 9500 µg a.s./L may be considered an outlier and exclusion of this endpoint from the geometric mean calculation, produces a value of 63 µg a.s./L.

|  |
| --- |
| **Review Comments:**  In accordance with the EFSA AGD, the risk assessment for algae should be performed considering the ErC50, where possible.  The zRMS is of the opinion that the lowest endpoint of ErC50 = 0.146 mg a.s./L for *N. pelliculosa*, given in the LoEP, should be considered for the risk assessment.  In addition, a refined risk assessment for algae based geometric mean calculation is not required, as safe use can be concluded using FOCUS Step 3 values. |

**Formulated product (BAS 736 00 F)**

The toxicity tests with representative aquatic species were conducted with the formulation BAS 736 00 F.

### Risk assessment

The evaluation of the risk for aquatic 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 (EFSA Aquatic GD) 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 EFSA AGD, risk assessment for algae and higher aquatic plants was performed considering only the more relevant endpoint “growth rate” (ErC50) where possible.

~~Furthermore, according to the EFSA Aquatic GD, the risk to aquatic life-stages of amphibians shall be addressed. In general, regarding the aquatic risk assessment, several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday S. and Thompson H., 2012, Weltje et al., 2013). Based on these extensive data reviews, it can be concluded that the acute and chronic risk to aquatic life stages of amphibians can be addressed by the currently requested and conducted risk assessment for aquatic organisms. This is also acknowledged in the Aquatic Guidance Document (EFSA, 2013).~~

**Acceptability of risk for fluxapyroxad (BAS 700 F)**

The EU agreed studies were used for risk assessment on aquatic organisms for the active substance fluxapyroxad and its relevant metabolites.

~~Besides the studies on the standard invertebrate test species~~ *~~D. magna~~*~~, acute and chronic studies using the active substance fluxapyroxad were conducted on the marine crustacean species~~ *~~A bahia.~~* ~~Testing on marine crustaceans~~~~is not a data requirement in the EU, however, the acute endpoints for~~ *~~A. bahia~~* ~~is also considered for the risk assessment as it is an EU agreed endpoint.~~ ~~Furthermore, an acute study on the marine bivalve mollusk species~~ *~~C. virginica~~* ~~has been performed with fluxapyroxad. The acute endpoint obtained for~~ *~~C. virginica~~* ~~provides the lowest endpoint for aquatic invertebrates and is thus also considered in the following ETR calculations.~~

~~To address chronic risk to fish, the recalculated endpoint for~~ *~~P. promelas~~* ~~has been used (for details please refer to justification of endpoints).~~

The relevant worst-case predicted environmental concentrations in surface water bodies (PECsw and PECsed), regulatory acceptable concentrations (RAC) for aquatic organisms and the resulting PEC/RAC ratios (ETR) for the single and twofold application of fluxapyroxad in ‘winter and spring cereals’ are given per intended use, each organism group and are presented in Table 9.5‑4 and Table 9.5‑5. Worst-case PEC values for either single or twofold application are considered in a risk envelope approach. For details on the PEC calculations please refer to Part B, Section 8.9.

**Table 9.5‑4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluxapyroxad for each organism group based on standard worst-case calculations following single and twofold application (1x and 2x 100 g a.s./ha) of BAS 736 00 F in ‘winter cereals’**

| **Group** |  | **Fish acute** | **Fish prolonged** | | **~~Fish prolonged~~** | **~~Inverteb. acute~~** | **~~Aquatic crustaceans acute~~** | **Aquatic gastropod molluscs acute** | **Inverteb. prolonged** | **Algae** | **Aquatic plants** | **~~Higher tier information~~** | **Group** | **Sed. dwell. prolonged** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test species** |  | ***C. carpio*** | ***P. promelas*** | | ***~~P. promelas~~*** | ***~~D. magna~~*** | ***~~A. bahia~~*** | ***C. virginica*** | ***D. magna*** | ***P. subcapitata*** | ***L. gibba*** | ***~~Fish acute:~~*** ~~SSD based on 96 h NOECs for 5 fish species~~ | **Test species** | ***C. riparius*** |
| **Endpoint** |  | LC50 | NOEC | | ~~NOEC~~ | ~~EC~~~~50~~ | ~~LC~~~~50~~ | EC50 | NOEC | ErC50 | ErC50 | ~~HC~~~~5~~ | **Endpoint** | NOEC |
| **(µg/L)** |  | 290 | 35.9 | | ~~67.6~~ | ~~6780~~ | ~~6100~~ | ~~> 2200~~  1100 | 500 | 700 | > 3430 | ~~148~~ | **(µg/kg)** | 75900 |
| **AF** |  | 100 | 10 | | ~~10~~ | ~~100~~ | ~~100~~ | 100 | 10 | 10 | 10 | ~~3~~ | **AF** | 10 |
| **RAC (µg/L)** |  | 2.9 | 3.59 | | ~~6.76~~ | ~~67.8~~ | ~~61~~ | ~~> 22~~ 11 | 50 | 70 | > 343 | ~~49.33~~ | **RAC (µg/kg)** | 7590 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)#** | **PEC/RAC (= ETR)** | | | | | | | | | | | **PEC gl-max (µg/kg)** | **PEC/RAC (= ETR)** |
| **Step 1** |  |  | |  | | | | | | | |  |  |  |
|  | 36.780 | **13** | **10.2** | | **~~5.4~~** | ~~0.5~~ | ~~0.6~~ | **~~< 1.7~~ 3.3** | 0.7 | 0.5 | < 0.1 | ~~0.7~~ | 244.341 | 0.03 |
| **Step 2** |  |  | |  | | | | | | | |  |  |  |
| N-Europe | 6.323 | **2.2** | **1.8** | | ~~0.9~~ | -- | -- | ~~< 0.3~~ 0.6 | -- | -- | -- | -- | 41.943 | -- |
| S-Europe | 11.641 | **4.0** | **3.2** | | **~~1.7~~** | -- | -- | ~~< 0.5~~ **1.1** | -- | -- | -- | -- | 78.134 | -- |
| **Step 3** |  |  | |  | | | | | | | |  |  |  |
| D3 ditch | 0.634 | 0.2 | 0.18 | | ~~0.09~~ | -- | -- | 0.06 | -- | -- | -- | -- | 0.379 | -- |
| D4 pond | 0.440 | 0.2 | 0.12 | | ~~0.07~~ | -- | -- | 0.04 | -- | -- | -- | -- | 3.181 | -- |
| D4 stream | 0.582 | 0.2 | 0.16 | | ~~0.09~~ | -- | -- | 0.05 | -- | -- | -- | -- | 1.065 | -- |
| D5 pond | 0.338 | 0.1 | 0.09 | | ~~0.05~~ | -- | -- | 0.03 | -- | -- | -- | -- | 3.516 | -- |
| D5 stream | 0.549 | 0.2 | 0.15 | | ~~0.08~~ | -- | -- | 0.05 | -- | -- | -- | -- | 0.643 | -- |
| R1 pond | 0.167 | 0.06 | 0.05 | | ~~0.02~~ | -- | -- | 0.015 | -- | -- | -- | -- | 1.313 | -- |
| R1 stream | 0.956 | 0.3 | 0.27 | | ~~0.1~~ | -- | -- | 0.09 | -- | -- | -- | -- | 1.299 | -- |
| R3 stream | 1.535 | 0.5 | 0.43 | | ~~0.2~~ | -- | -- | 0.14 | -- | -- | -- | -- | 1.442 | -- |
| R4 stream | 0.845 | 0.3 | 0.24 | | ~~0.1~~ | -- | -- | 0.08 | -- | -- | -- | -- | 0.938 | -- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in bold

# PEC values are derived from calculation for either single or twofold application of BAS 736 00 F in winter cereals in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended use of fluxapyroxad in ‘winter cereals’ at 1x and 2x 100 g a.s./ha, the calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and Step 1 - 3 PECsw and PECsed values. Therefore, no further assessment is necessary.

**Table 9.5‑5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluxapyroxad for each organism group based on standard worst-case calculations following single and twofold application (1x and 2x 100 g a.s./ha) of BAS 736 00 F in ‘spring cereals’**

| **Group** |  | **Fish acute** | **Fish prolonged** | | **~~Fish prolonged~~** | **~~Inverteb. acute~~** | **~~Aquatic crustaceans acute~~** | **Aquatic gastropod molluscs acute** | **Inverteb. prolonged** | **Algae** | **Aquatic plants** | **~~Higher tier information~~** | **Group** | **Sed. dwell. prolonged** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test species** |  | ***C. carpio*** | ***P. promelas*** | | ***~~P. promelas~~*** | ***~~D. magna~~*** | ***~~A. bahia~~*** | ***C. virginica*** | ***D. magna*** | ***P. subcapitata*** | ***L. gibba*** | ***~~Fish acute:~~*** ~~SSD based on 96 h NOECs for 5 fish species~~ | **Test species** | ***C. riparius*** |
| **Endpoint** |  | LC50 | NOEC | | ~~NOEC~~ | ~~EC~~~~50~~ | ~~LC~~~~50~~ | EC50 | NOEC | ErC50 | ErC50 | ~~HC~~~~5~~ | **Endpoint** | NOEC |
| **(µg/L)** |  | 290 | 35.9 | | ~~67.6~~ | ~~6780~~ | ~~6100~~ | ~~> 2200~~  1100 | 500 | 700 | > 3430 | ~~148~~ | **(µg/kg)** | 75900 |
| **AF** |  | 100 | 10 | | ~~10~~ | ~~100~~ | ~~100~~ | 100 | 10 | 10 | 10 | ~~3~~ | **AF** | 10 |
| **RAC (µg/L)** |  | 2.9 | 3.59 | | ~~6.76~~ | ~~67.8~~ | ~~61~~ | ~~> 22~~ 11 | 50 | 70 | > 343 | ~~49.33~~ | **RAC (µg/kg)** | 7590 |
| **FOCUS Scenario** | **PEC gl-max (µg/L)#** | **PEC/RAC (= ETR)** | | | | | | | | | | | **PEC gl-max (µg/kg)** | **PEC/RAC (= ETR)** |
| **Step 1** |  |  | |  | | | | | | | |  |  |  |
|  | 36.780 | **13** | **10.2** | | **~~5.4~~** | 0.5 | 0.6 | **~~< 1.7~~ 3.3** | 0.7 | 0.5 | < 0.1 | ~~0.7~~ | 244.341 | 0.03 |
| **Step 2** |  |  | |  | | | | | | | |  |  |  |
| N-Europe | 6.323 | **2.2** | **1.8** | | ~~0.9~~ | -- | -- | ~~< 0.3~~ 0.6 | -- | -- | -- | -- | 41.943 | -- |
| S-Europe | 11.641 | **4.0** | **3.2** | | **~~1.7~~** | -- | -- | ~~< 0.5~~ **1.1** | -- | -- | -- | -- | 78.134 | -- |
| **Step 3** |  |  | |  | | | | | | | |  |  |  |
| D3 ditch | 0.634 | 0.2 | 0.18 | | ~~0.09~~ | -- | -- | 0.06 | -- | -- | -- |  | 0.381 | -- |
| D4 pond | 0.576 | 0.2 | 0.16 | | ~~0.09~~ | -- | -- | 0.05 | -- | -- | -- |  | 4.100 | -- |
| D4 stream | 0.724 | 0.2 | 0.20 | | ~~0.1~~ | -- | -- | 0.07 | -- | -- | -- |  | 1.404 | -- |
| D5 pond | 0.301 | 0.1 | 0.08 | | ~~0.04~~ | -- | -- | 0.03 | -- | -- | -- |  | 3.189 | -- |
| D5 stream | 0.536 | 0.2 | 0.15 | | ~~0.08~~ | -- | -- | 0.05 | -- | -- | -- |  | 0.569 | -- |
| R4 stream | 0.895 | 0.3 | 0.25 | | ~~0.1~~ | -- | -- | 0.08 | -- | -- | -- |  | 0.982 | -- |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in bold

# PEC values are derived from calculation for either single or twofold application of BAS 736 00 F in spring cereals in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended use of fluxapyroxad in ‘spring cereals’ at 1x and 2x 100 g a.s./ha, the calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and Step 1 - 3 PECsw and PECsed values. Therefore, no further assessment is necessary.

**Metabolites of fluxapyroxad (BAS 700 F)**

The ETR ratios for the relevant metabolites of fluxapyroxad are presented in Table 9.5‑6 for the intended uses of BAS 736 00 F in ‘winter cereals’ (covering ‘spring cereals’) for each aquatic organism group. Only the worst-case PEC values from either single or twofold application are considered for calculations.

**Table 9.5‑6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of fluxapyroxad for each organism group based on standard worst-case PEC calculations following single and twofold application (1x and 2x 100 g a.s./ha) of BAS 736 00 F in ‘winter cereals’ (covering ‘spring cereals’)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** |
| **AF** | | 100 | 100 | 10 |
| **M700F001** | | | | |
| Endpoint (µg/L) | | LC50 | EC50 | ErC50 |
| > 100000 | > 100000 | 36310 |
| RAC (µg/L) | | > 1000 | > 1000 | 3631 |
| **FOCUS Scenario** | **PEC gl-max, sw (µg/L)** | **PEC/RAC ratio (= ETR)** | | |
| **Step 1** |  |  | | |
|  | 7.153 | < 0.007 | < 0.007 | 0.002 |
| **M700F002** | | | | |
| Endpoint (µg/L) | | LC50 | EC50 | ErC50 |
| > 100000 | > 100000 | 26520 |
| RAC (µg/L) | | > 1000 | > 1000 | 2652 |
| **FOCUS Scenario** | **PEC gl-max, sw (µg/L)** | **PEC/RAC ratio (= ETR)** | | |
| **Step 1** |  |  | | |
|  | 19.816 | < 0.02 | < 0.02 | 0.0007 |
| **M700F007** | | | | |
| Endpoint (µg/L) | | LC50 | EC50 | ErC50 |
| > 100000 | > 100000 | > 100000 |
| RAC (µg/L) | | > 1000 | > 1000 | > 10000 |
| **FOCUS Scenario** | **PEC gl-max, sw (µg/L)** | **PEC/RAC ratio (= ETR)** | | |
| **Step 1** |  |  | | |
|  | 5.564 | < 0.006 | < 0.006 | < 0.0006 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio

For the intended single and twofold application of BAS 736 00 F in ‘winter cereals’ and ‘spring cereals’ at 1x and 2x 100 g a.s./ha, the calculated PEC/RAC ratios for the fluxapyroxad metabolites indicate an acceptable risk for all groups of aquatic organisms based on standard assumptions. Therefore, no further assessment is necessary.

**~~Refined Risk Assessment for fluxapyroxad– Tier-2B: Species Sensitivity Distribution (SSD)~~**

~~For fluxapyroxad and based on the available regulatory acceptable concentrations (RAC) from standard test organisms, fish is one order of magnitude the most sensitive group to this fungicide and hence chosen for further risk assessment refinement. A species sensitivity distribution (SSD) is then used to refine the RAC for this group of aquatic organisms since according to the Tier 2B approach in the EFSA AGD (2013), data on the toxicity of fluxapyroxad is available at is obtained from at least fish species.~~

~~According to the EFSA AGD (2013), it is recommended to use chronic endpoints NOEC/LC10 to construct the SSD for fish, since a higher protection level is desired for vertebrates than for invertebrates and plants. Five NOECs were obtained from acute studies performed to fulfill different zonal requirements under the guideline OECD 203 and with an exposure duration of 96 h.~~

~~The median hazard concentration to 5% of the tested species (HC5) derived from the SSD may subsequently be used in combination with an AF of 3 to calculate the SSD-RAC used for comparison with FOCUS PEC~~~~sw~~ ~~values.~~

**~~Table 9.5‑7: Overview on the fish toxicity endpoints available for fluxapyroxad and used for the species sensitivity distribution (SSD) analysis~~**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **~~Species~~** | **~~Guideline~~** | **~~Exposure type~~** | **~~Exposure duration~~** | **~~NOEC~~**  **~~[µg a.s./L]~~** | **~~BASF DocID~~** |
| *~~Oncorhynchus mykiss~~* | ~~OECD 203~~ | ~~static~~ | ~~96 h~~ | **~~212~~** | ~~2007/1057974 (v1.0)~~ |
| *~~Cyprinus carpio~~* | ~~OECD 203~~ | ~~semi-static~~ | ~~96 h~~ | **~~168~~** | ~~2008/1064998~~  ~~+NOEC from 2008/1064998 US OECD Summary (v1.0)~~ |
| *~~Lepomis macrochirus~~* | ~~OECD 203~~ | ~~static~~ | ~~96 h~~ | **~~572~~** | ~~2008/1010605~~ |
| *~~Promelas promelas~~* | ~~OECD 203~~ | ~~static~~ | ~~96 h~~ | **~~327~~** | ~~2009/1025414~~ |
| *~~Cyprinodon variegatus~~* | ~~OECD 203~~ | ~~static~~ | ~~96 h~~ | **~~370~~** | ~~2008/7015417~~ |

**~~SSD calculation~~**

~~SSD calculations were performed using the using the open-source R software and the R package “ssdtools” (Thorley and Schwarz, 2018) and an associated proprietary SSD Tool application (Dalgarno, 2018). The R script used to derive the SSD (please refer to the appendix of this chapter) and the results of SSD calculations are shown below. The NOEC values derived from the studies listed in Table 9.5‑7 were inserted in the software and several statistical distributions and models evaluated. It is generally recommendable to choose a statistical distribution from different models with goodness of fit analysis to determine the most appropriate model for a data set (EFSA, 2013). The “ssdtools” package allows a user to fit gamma, Gompertz, log-Gumbel, log-logistic, log-normal, and Weibull distributions to the data and to decide about the appropriate distribution under consideration of the model fit. For this fit, the Burr function was also included.~~

**~~Goodness-of-fit statistics~~**

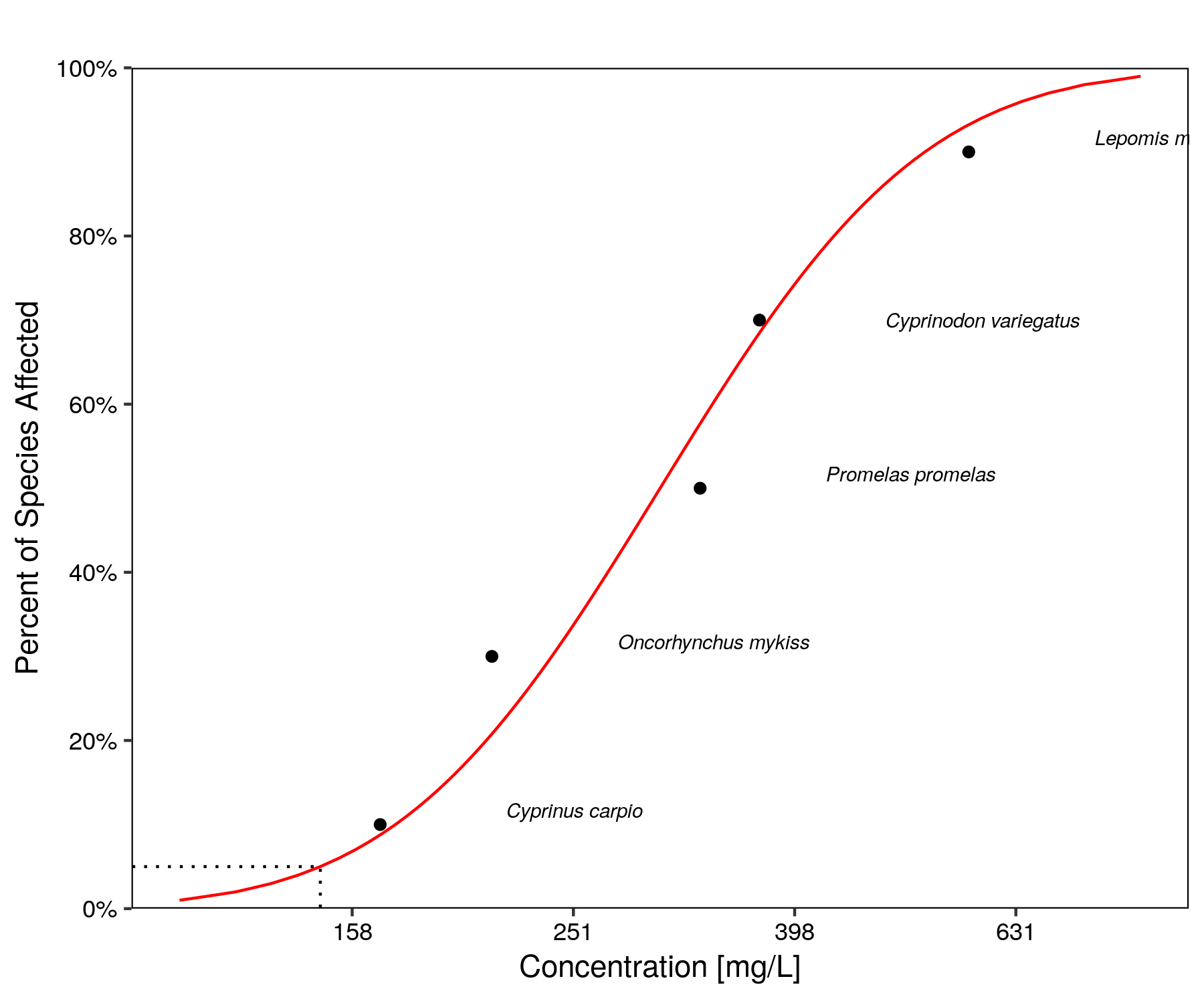
~~The selected distributions were ranked according to goodness-of-fit (Table 9.5‑8) according to the lowest AIC~~~~C~~ ~~value (Akaike’s information criterion corrected for sample size). This criterion is recommended for selecting the best distribution. Similarly, the lowest BIC value (Bayesian information criterion) indicates the model with the best fit. Thus, the Log-normal distribution provides the best-fitting model AIC~~~~c~~ ~~= 66.8, and BIC = 66) and chosen to further obtain the HC~~~~5~~~~.~~

**~~Table 9.5‑8: Overview on goodness-of-fit for selected distributions ordered by AICc then AIC,~~ *~~i.e.~~*~~, the best model fit is listed on top of the table. \*~~**

| **~~Distribution~~** | **~~aic~~** | **~~aicc~~** | **~~bic~~** | **~~delta~~** | **~~weight~~** |
| --- | --- | --- | --- | --- | --- |
| **~~lnorm~~** | ~~66.8~~ | ~~72.8~~ | ~~66.0~~ | ~~0.000~~ | ~~0.223~~ |
| ~~gamma~~ | ~~66.9~~ | ~~72.9~~ | ~~66.2~~ | ~~0.118~~ | ~~0.210~~ |
| ~~lgumbel~~ | ~~66.9~~ | ~~72.9~~ | ~~66.2~~ | ~~0.128~~ | ~~0.209~~ |
| ~~weibull~~ | ~~67.3~~ | ~~73.3~~ | ~~66.5~~ | ~~0.443~~ | ~~0.179~~ |
| ~~Llogis~~ | ~~67.3~~ | ~~73.3~~ | ~~66.5~~ | ~~0.454~~ | ~~0.178~~ |
| ~~burrlII3~~ | ~~68.9~~ | ~~92.9~~ | ~~67.8~~ | ~~20.1~~ | ~~0~~ |

~~\* The columns in the goodness of fit table are the Akaike’s Information Criterion (aic), Akaike’s Information Criterion corrected for sample size (aicc), Bayesian Information Criterion (bic), the AICc difference (delta) and the AICc based Akaike weight (weight). The goodness of fit table is ordered by AICc, the best model fit is listed on top of the table.~~

**~~Figure 9.5‑1: Species sensitivity distribution (SSD) curve derived from 96-h NOEC values for five fish species. Empirical NOECs (black dots) and predicted Cumulative Distribution Function (CDF, red line), based on fluxapyroxad toxicity data for 5 fish species (calculated with the software R; log-normal distribution). The HC~~~~5~~ ~~value is shown at the intersection of the two dashed lines on the red curve.~~**

~~~~

**~~Estimation of hazardous concentrations~~**

~~The SSD constructed for fish resulted in a median HC~~~~5~~ ~~of~~ **~~148 µg fluxapyroxad/L~~**~~. According to the recommendation given in the EFSA AGD (2013), an AF of 3 should be applied on the median HC~~~~5~~ ~~from an SSD constructed with acute NOEC values for fish for derivation of an SSD-RAC. Following this approach, an acute SSD-RAC for fish of~~ **~~49.33 µg a.s./L~~** ~~can be derived~~

**~~HC~~~~5~~ ~~results based on acute (96 h) NOEC data for 5 fish species~~**

|  |  |
| --- | --- |
|  | **~~Hazardous Concentration [µg a.s./L]~~** |
| **~~Median HC~~~~5~~ ~~(lower – upper confidence intervals)~~** | **~~148 (89.1- 282)~~** |
| **~~AF = 3~~** | |
| **~~SSD-RAC~~** | **~~49.33~~** |

**Acceptability of risk for azoxystrobin**

The EU agreed studies were used for risk assessment on aquatic organisms for the active substance azoxystrobin and its relevant metabolites.

~~Considering toxicity to algae, a recalculated geomean differing from the EFSA conclusion for azoxystrobin was used in the risk assessment (~~*~~i.e.,~~* ~~Geomean E~~~~b~~~~C~~~~50~~ ~~= 63 µg a.s./L instead of 262 µg a.s./L). For details, please refer to 9.5.1.1~~

The relevant worst-case predicted environmental concentrations in surface water bodies (PECsw and PECsed), regulatory acceptable concentrations (RAC) for aquatic organisms and the resulting PEC/RAC ratios (ETR) for the single and twofold application of fluxapyroxad in ‘cereals’ (covering spring and winter cereals) are given per intended use and each organism group and are presented in Table 9.5‑9 and Table 9.5‑10. Worst-case PEC values for either winter or spring cereals are considered in a risk envelope approach. For details on the PEC calculations please refer to Part B, Section 8.9.

**Table 9.5‑9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on worst-case calculations following single and twofold application (1x and 2x 150 g a.s./ha) of BAS 736 00 F in ‘winter cereals’**

| **Group** |  | **Fish acute** | **Fish prolonged** | | **Inverteb. acute** | | **Inverteb. acute** | | **Inverteb. prolonged** | **~~Algae~~** | **Algae** | **Aquatic plants** | **Sed. dwell. prolonged** | **Higher Tier information** | **Group** | | | **Sed. dwell. prolonged** | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test species** |  | ***O. mykiss*** | ***P. promelas*** | | ***M. bahia*** | | ***D. magna*** | | ***D. magna*** | **~~Geomean (3 algal species)~~** | ***N. pelliculosa*** | ***L. gibba*** | ***C. riparius*** | **Mesocosm** | **Test species** | | | ***C. riparius*** | |
| **Endpoint** |  | LC50 | NOEC | | EC50 | | EC50 | | NOEC | ~~EC~~~~50~~ | ErC50 | EC50 | NOEC | NOAEC | **Endpoint** | | | NOEC | |
| **(µg/L)** |  | 470 | 147 | | 55 | | 230 | | 44 | ~~63~~ | 146 | 3200 | 800 | 10 | **(µg/kg)** | | | 23000 | |
| **AF** |  | 100 | 10 | | 100 | | 100 | | 10 | ~~10~~ | 10 | 10 | 10 | 3 | **AF** | | | 10 | |
| **RAC (µg/L)** |  | 4.7 | 14.7 | | 0.55 | | 2.3 | | 4.4 | ~~6.3~~ | 14.6 | 320 | 80 | 3.3 | **RAC (µg/kg)** | | | 2300 | |
| **FOCUS**  **Scenario** | **PEC gl-max (µg/L)#** | **PEC/RAC (= ETR)** | | | | | | | | | | | | | **PEC gl-max (µg/kg)#** | | | **PEC/RAC (= ETR)** | |
| **Step 1** |  |  | |  | |  | |  | | | | | | | |  |  | |  |
|  | 68.433 | **15** | **4.7** | | **124.4** | | **30** | | **16** | **~~11~~** | **4.7** | 0.2 | 0.9 | **21** | 263.653 | | | 0.1 | |
| **Step 2** |  |  | |  | |  | |  | | | | | | | |  |  | |  |
| N-Europe | 11.290 | **2.4** | 0.8 | | **20.5** | | **4.9** | | **2.6** | **~~1.8~~** | **0.8** | -- | -- | **3.4** | 43.330 | | | -- | |
| S-Europe | 20.838 | **4.4** | **1.4** | | **37.9** | | **9.1** | | **4.7** | **~~3.3~~** | **1.4** | -- | -- | **6.3** | 80.630 | | | -- | |
| **Step 3** |  |  | |  | |  | |  | | | | | | | |  |  | |  |
| D3 ditch | ~~0.951~~  0.950 | 0.2 | 0.06 | | **1.72** | | 0.4 | | 0.2 | ~~0.2~~ | 0.065 | -- | -- | 0.3 | ~~0.574~~  0.523 | | | -- | |
| D4 pond | ~~0.556~~  0.563 | 0.1 | 0.04 | | **1.02** | | 0.2 | | 0.1 | ~~0.09~~ | 0.04 | -- | -- | 0.2 | ~~3.438~~  3.466 | | | -- | |
| D4 stream | 0.793 | 0.2 | 0.05 | | **1.44** | | 0.3 | | 0.2 | ~~0.1~~ | 0.05 | -- | -- | 0.2 | ~~1.316~~  1.321 | | | -- | |
| D5 pond | ~~0.197~~  0.198 | 0.04 | 0.01 | | 0.36 | | 0.09 | | ~~0.04~~  0.05 | ~~0.03~~ | 0.01 | -- | -- | 0.06 | ~~1.910~~  1.784 | | | -- | |
| D5 stream | 0.762 | 0.2 | 0.05 | | **1.39** | | 0.3 | | 0.2 | ~~0.1~~ | 0.05 | -- | -- | 0.2 | ~~0.391~~  0.390 | | | -- | |
| R1 pond | ~~0.276~~  0.278 | 0.06 | 0.02 | | 0.51 | | 0.1 | | 0.06 | ~~0.04~~ | 0.02 | -- | -- | 0.08 | ~~1.574~~  1.600 | | | -- | |
| R1 stream | ~~1.620~~  1.633 | 0.3 | 0.1 | | **2.97** | | 0.7 | | 0.4 | ~~0.3~~ | 0.11 | -- | -- | 0.5 | ~~1.767~~  1.762 | | | -- | |
| R3 stream | ~~3.111~~  3.128 | 0.7 | 0.2 | | **5.69** | | **1.4** | | 0.7 | ~~0.5~~ | 0.21 | -- | -- | 0.9 | ~~2.177~~  2.184 | | | -- | |
| R4 stream | ~~2.841~~  1.603 | ~~0.6~~  0.3 | ~~0.2~~  0.1 | | **2.91** | | **~~1.2~~**  0.7 | | ~~0.6~~  0.4 | ~~0.5~~  ~~0.3~~ | 0.11 | -- | -- | ~~0.9~~  0.5 | ~~1.612~~  1.383 | | | -- | |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in bold; n.c.: not calculated

# Worst-case PECs are derived from either single or twofold application in a risk envelope approach.For details, please refer to Part B, Section 8.9.

\* Not calculated, drainage PECs for single application are covered by 2-fold application.

For the intended use of azoxystrobin in ‘winter cereals’ at 1x and 2x 150 g a.s./ha, the calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and Step 1 - 3 PECsw and PECsed values except for acute risk to invertebrates. Therefore, higher tier studies have been conducted (*i.e.,* mesocosm study). Considering this higher tier information (*i.e.,* mesocosm RAC of 3.3 a.s./L) PEC/RAC ratios indicate an acceptable risk for aquatic invertebrate. Therefore, no further assessment is necessary.

**Table 9.5‑10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for azoxystrobin for each organism group based on worst-case calculations following single and twofold application (1x and 2x 150 g a.s./ha) of BAS 736 00 F in ‘spring cereals’**

| **Group** |  | **Fish acute** | **Fish prolonged** | | **Inverteb. acute** | | **Inverteb. acute** | | **Inverteb. prolonged** | **~~Algae~~** | **Algae** | **Aquatic plants** | **Sed. dwell. prolonged** | **Higher Tier information** | **Group** | | | **Sed. dwell. prolonged** | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test species** |  | ***O. mykiss*** | ***P. promelas*** | | ***M. bahia*** | | ***D. magna*** | | ***D. magna*** | **~~Geomean (3 algal species)~~** | ***N. pelliculosa*** | ***L. gibba*** | ***C. riparius*** | **Mesocosm** | **Test species** | | | ***C. riparius*** | |
| **Endpoint** |  | LC50 | NOEC | | EC50 | | EC50 | | NOEC | ~~EC~~~~50~~ | ErC50 | EC50 | NOEC | NOAEC | **Endpoint** | | | NOEC | |
| **(µg/L)** |  | 470 | 147 | | 55 | | 230 | | 44 | ~~63~~ | 146 | 3200 | 800 | 10 | **(µg/kg)** | | | 23000 | |
| **AF** |  | 100 | 10 | | 100 | | 100 | | 10 | ~~10~~ | 10 | 10 | 10 | 3 | **AF** | | | 10 | |
| **RAC (µg/L)** |  | 4.7 | 14.7 | | 0.55 | | 2.3 | | 4.4 | ~~6.3~~ | 14.6 | 320 | 80 | 3.3 | **RAC (µg/kg)** | | | 2300 | |
| **FOCUS**  **Scenario** | **PEC gl-max (µg/L)#** | **PEC/RAC (= ETR)** | | | | | | | | | | | | | **PEC gl-max (µg/kg)#** | | | **PEC/RAC (= ETR)** | |
| **Step 1** |  |  | |  | |  | |  | | | | | | | |  |  | |  |
|  | 68.433 | **15** | **4.7** | | **124.4** | | **30** | | **16** | **11** | **4.7** | 0.2 | 0.9 | **21** | 263.653 | | | 0.1 | |
| **Step 2** |  |  | |  | |  | |  | | | | | | | |  |  | |  |
| N-Europe | 11.290 | **2.4** | 0.8 | | **20.5** | | **4.9** | | **2.6** | **1.8** | 0.8 | -- | -- | **3.4** | 43.330 | | | -- | |
| S-Europe | 20.838 | **4.4** | **1.4** | | **37.9** | | **9.1** | | **4.7** | **3.3** | **1.4** | -- | -- | **6.3** | 80.630 | | | -- | |
| **Step 3** |  |  | |  | |  | |  | | | | | | | |  |  | |  |
| D3 ditch | ~~0.951~~  0.950 | 0.2 | 0.06 | | **1.72** | | 0.4 | | 0.2 | 0.2 | 0.065 | -- | -- | 0.3 | ~~0.550~~  0.526 | | | -- | |
| D4 pond | ~~0.689~~  0.695 | 0.1 | 0.05 | | **1.26** | | 0.3 | | 0.2 | 0.1 | 0.05 | -- | -- | 0.2 | ~~4.228~~  4.227 | | | -- | |
| D4 stream | 0.778 | 0.2 | 0.05 | | **1.41** | | 0.3 | | 0.2 | 0.1 | 0.05 | -- | -- | 0.2 | ~~1.624~~  1.612 | | | -- | |
| D5 pond | ~~0.194~~  0.196 | 0.04 | 0.01 | | 0.36 | | ~~0.08~~  0.09 | | 0.04 | 0.03 | 0.01 | -- | -- | 0.06 | ~~1.816~~  1.707 | | | -- | |
| D5 stream | 0.801 | 0.2 | 0.05 | | **1.46** | | 0.3 | | 0.2 | 0.1 | 0.05 | -- | -- | 0.2 | ~~0.381~~  0.384 | | | -- | |
| R4 stream | ~~3.027~~  1.771 | ~~0.6~~  0.4 | ~~0.2~~  0.1 | | **3.22** | | **~~1.3~~**  0.8 | | ~~0.7~~  0.4 | ~~0.5~~  0.3 | 0.12 | -- | -- | ~~0.9~~  0.5 | ~~2.379~~  1.439 | | | -- | |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in bold

# Worst-case PECs are derived from either single or twofold application in a risk envelope approach.For details, please refer to Part B, Section 8.9.

For the intended use of azoxystrobin in ‘spring cereals’ at 1x and 2x 150 g a.s./ha, the calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and Step 1 - 3 PECsw and PECsed values except for acute risk to invertebrates. Therefore, higher tier studies have been conducted (*i.e.,* mesocosm study). Considering this higher tier information (*i.e.* mesocosm RAC of 3.3 a.s./L) PEC/RAC ratios indicate an acceptable risk for aquatic invertebrate. Therefore, no further assessment is necessary.

**Metabolites of azoxystrobin**

The ETR ratios for the relevant metabolites of azoxystrobin are presented in Table 9.5‑11 for the intended uses of BAS 736 00 F in ‘cereals’ for each aquatic organism group. Only the worst-case PEC values from either single or twofold application in ‘winter or spring cereals’ are considered for calculations, covering all proposed uses.

**Table 9.5‑11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of azoxystrobin for each organism group based on standard worst-case PEC calculations following single and twofold application (1x and 2x 150 g a.s./ha) of BAS 736 00 F in ‘cereals’ (covering all proposes uses)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | | **Fish acute** | **Inverteb. acute** | **Algae** |
| **Test species** | | ***O. mykiss*** | ***D. magna*** | ***P. subcapitata*** |
| **AF** | | 100 | 100 | 10 |
| **R234886** | | | | |
| **Endpoint (µg/L)** | | LC50 | EC50 | EbC50 |
| > 150000 | > 180000 | 47000 |
| **RAC (µg/L)** | | > 1500 | > 1800 | 470 |
| **FOCUS**  **Scenario** | **PEC gl-max, sw (µg/L)** | PEC/RAC ratio (= ETR) | | |
| **Step 1** |  |  | | |
|  | 44.135 | < 0.03 | < 0.03 | 0.09 |
| **R402173** | | | | |
| **Endpoint (µg/L)** | | LC50 | EC50 | EbC50 |
| 62000 | > 100000 | 67000 |
| **RAC (µg/L)** | | 620 | > 1000 | 670 |
| **FOCUS**  **Scenario** | **PEC gl-max, sw (µg/L)** | PEC/RAC ratio (= ETR) | | |
| **Step 1** |  |  | | |
|  | 11.361 | 0.02 | < 0.01 | 0.02 |
| **R401553** | | | | |
| **Endpoint (µg/L)** | | LC50 | EC50 | EbC50 |
| > 120000 | > 120000 | > 120000 |
| **RAC (µg/L)** | | > 1200 | > 1200 | > 12000 |
| **FOCUS**  **Scenario** | **PEC gl-max, sw (µg/L)** | PEC/RAC ratio (= ETR) | | |
| **Step 1** |  |  | | |
|  | 15.567 | < 0.01 | < 0.01 | < 0.001 |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio

For the intended single and twofold application of BAS 736 00 F in ‘winter cereals’ and ‘spring cereals’ at 1x and 2x 150 g a.s./ha, the calculated PEC/RAC ratios for the azoxystrobin metabolites indicate an acceptable risk for all groups of aquatic organisms based on standard assumptions. Therefore, no further assessment is necessary.

**Formulation risk assessment for BAS 736 00 F**

In the following a mixture toxicity risk assessment for the formulated product is presented, which was conducted in accordance with the EFSA AGD (2013. The EFSA Aquatic GD proposes that measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used. To determine the respective formulation effect, EFSA proposes to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity (LC50 mix-CA / EC50 mix-CA) by the measured mixture toxicity (LC50 PPP / EC50 PPP). If the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement. Respective MDR calculations for BAS 736 00 F are presented in Table 9.5‑12.

**Table 9.5‑12: Comparison of the measured toxicity of the formulated product BAS 736 00 F and the calculated formulation toxicity based on the data of the active substances fluxapyroxad and azoxystrobin**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Test species** | **Test system** | **Endpoint** | **Measured toxicity of the a.s. (ECx a.s.)  [µg a.s./L]** | | **Measured toxicity of BAS 736 00 F**  **(ECx PPP)  [µg product/L]** | **Calculated formulation toxicity  (ECx mix-CA) [µg mixture/L] \*** | **Ratio (MDR) (ECx mix-CA / ECx PPP)** |
| *O. mykiss* | acute | 96-h LC50 | fluxapyroxad | 546# | 2830 (328.2 µg a.s./L) | 4292.3  (497.7 µg a.s./L) | 1.5 |
| azoxystrobin | 470 |
| *D. magna* | acute | 48-h EC50 | fluxapyroxad | 6780 | 1570  (182.1 µg a.s./L) | 3232.8  (374.9 µg a.s./L) | 2.1 |
| azoxystrobin | 230 |
| *P. subcapitata* | - | EC50 | fluxapyroxad | 700 | 4190  (485.9 µg a.s./L) | 2239.9  (259.7 µg a.s./L) | 0.5 |
| azoxystrobin | 183# |

PPP = Plant Protection Product; CA = concentration addition

**\*** The theoretical mixture toxicity of the formulation was re-calculated assuming concentration addition based on the measured toxicity data of the active substances and their nominal content within the formulation (*i.e.* 50 g fluxapyroxad/L and 75 g azoxystrobin/L) and the formulation density (1.078 kg/L).

# Endpoint for MDR diverging from endpoint used in the risk assessment in scope of comparability reasons.

The calculated MDR values are between 0.5 and 2.1 for all organisms, indicating that the formulation does not cause synergistic or antagonistic toxicity compared to the active substance but instead follows the expected toxicity for all groups of aquatic organisms (*i.e.* the CA model provides a reliable estimate of the toxicity of the given mixture).

With regard to the mixture risk assessment, the EFSA Aquatic GD further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance and the CA model provides a reliable estimate of the toxicity of the given mixture, a sufficient protection level might be achieved by simply basing the risk assessment on the toxicity data for that single ‘driver̕. Whether one a.s. is driving toxicity of the given mixture can be verified by the “Toxic Unit (TU)” approach. The EFSA Aquatic GD states that if more than 90% of the sum of toxic units calculated for the formulation comes from a single a.s., the risk assessment is sufficiently addressed by the risk assessment for the active substances. TU calculations for BAS 736 00 F are presented in Table 9.5‑13.

Table 9.5‑13: Toxic Unit calculations for BAS 736 00 F based on the concentration of the active substances fluxapyroxad and azoxystrobin

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Test**  **substance** | **Test system** | **Nominal content of a.s. in BAS 736 00 F [g/L]** | **Measured toxicity of the a.s. (EC50a.s. / NOECa.s.)  [µg a.s./L]** | **Toxic Unit (TU)** | **Toxic Unit [%]** |
| **Fish, acute** | fluxapyroxad | 96‑h LC50 | 50 | 546 | 91575 | 36.5 |
| *O. mykiss* |
| azoxystrobin | 96‑h LC50 | 75 | 470 | 159574 | 63.5 |
| *O. mykiss* |
|  | SUM TU |  |  |  | 251150 |  |
| **Fish,**  **prolonged** | fluxapyroxad | 33‑d NOEC | 50 | 67.6 | 739645 | 59.2 |
| *P. promelas* |
| azoxystrobin | 33‑d NOEC | 75 | 147 | 510204 | 40.8 |
| *P. promelas* |
|  | SUM TU |  |  |  | 1249849 |  |
| **Invertebrate, acute** | fluxapyroxad | 48‑h EC50 | 50 | 6780 | 7375 | 2.2 |
| *D. magna* |
| azoxystrobin | 48‑h EC50 | 75 | 230 | 326087 | **97.8** |
| *D. magna* |
|  | SUM TU |  |  |  | 333462 |  |
| **Invertebrate, prolonged** | fluxapyroxad | 21‑d NOEC | 50 | 500 | 100000 | 5.5 |
| *D. magna* |
| azoxystrobin | 21‑d NOEC | 75 | 44 | 1704545 | **94.5** |
| *D. magna* |
|  | SUM TU |  |  |  | 1804545 |  |
| **Algae** | fluxapyroxad | 72‑h / 11‑d ErC50 | 50 | 700 | 71429 | 14.8 |
| *P. subcapitata* |
| azoxystrobin | 72 h / 11 d ErC50 | 75 | 183 | 409836 | 85.2 |
| *P. subcapitata* |
|  | SUM TU |  |  |  | 481265 |  |

**Bold values**: Toxic units > 90% for a single a.s.

TU calculations for fish and algae indicate that none of the active substance solely accounts for the acute and chronic toxicity of the formulated product BAS 736 00 F. However, for aquatic invertebrates, azoxystrobin is driving the toxicity (*i.e.* 94.46%) of the formulated product. Therefore, in line with the EFSA Aquatic GD (2013), the acute and chronic risk assessment for aquatic invertebrates is sufficiently addressed by the risk assessment for the active substance that drives the toxicity

Based on the calculated MDRs (see Table 9.5‑12) it can be concluded that chronic studies on fish and invertebrates with the formulations are not required (product is not by a factor ≥10 acutely more toxic than the a.s.) according to the EFSA Aquatic GD (2013). Furthermore, as the formulation will break down rapidly once in the environment, no chronic exposure to the formulated product is expected.

Accordingly, an acute lower and higher tier risk assessment for fish and algae considering PECmix values and measured endpoints from formulation studies (recalculated to content of a.s.) are provided in Table 9.5‑14 to Table 9.5‑17.

**Table 9.5‑14: Aquatic organisms: acceptability of risk (PECmix/RACPPP < 1) for the formulation BAS 736 00 F for fish and algae group based on worst-case PECmix values following single and twofold application of BAS 736 00 F in ‘winter cereals’**

| **Group** |  | **Fish acute** | **Algae** | |
| --- | --- | --- | --- | --- |
| **Test species** |  | ***O. mykiss*** | ***P. subcapitata*** | |
| **Endpoint** |  | LC50 | ErC50 | |
| **(µg a.s./L)** |  | 328.2 | 485.9 | |
| **AF** |  | 100 | 10 | |
| **RAC (µg a.s./L)** |  | 3.282 | 48.59 | |
| **FOCUS**  **Scenario** | **PECmix-max (µg/L)#** | **PEC/RAC (= ETR)** | | |
| **Step 1** |  |  | |  |
|  | 105.213 | **32** | **2.2** | |
| **Step 2** |  |  | |  |
| N-Europe | 17.613 | **5.4** | 0.4 | |
| S-Europe | 32.479 | **9.9** | 0.7 | |
| **Step 3** |  |  | |  |
| D3 ditch | ~~1.585~~  1.584 | 0.5 | -- | |
| D4 pond | ~~0.996~~  1.003 | 0.3 | -- | |
| D4 stream | 1.375 | 0.4 | -- | |
| D5 pond | ~~0.535~~  0.536 | 0.2 | -- | |
| D5 stream | 1.311 | 0.4 | -- | |
| R1 pond | ~~0.443~~  0.445 | 0.1 | -- | |
| R1 stream | ~~2.576~~  2.589 | 0.8 | -- | |
| R3 stream | ~~4.646~~  4.663 | **1.4** | -- | |
| R4 stream | ~~3.686~~  2.448 | **~~1.1~~**  0.7 | -- | |
| Step 4 (10+10) | | | | |
| R3 stream | 2.129 | 0.65 | -- | |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in **bold**

# Worst-case PECs are derived from either single or twofold application in a risk envelope approach.For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 736 00 F in ‘winter cereals’ the calculated PECmix/RACPPP ratios indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and Step 1 - 3 PECsw mix values except for acute risk to fish at FOCUS stream scenario~~s~~ R3 ~~and R4~~.

Considering FOCUS Step 4 PEC values (10+10) for both active substances PEC/RAC ratios indicate an acceptable risk for fish.

~~Therefore, a refined risk assessment considering higher tier data for acute fish from fluxapyroxad is shown below (see Table 9.5‑15) in terms of using a risk quotient (RQ) approach for the critical stream scenarios.~~

**~~Table 9.5‑15: Risk quotient (RQ~~~~mix~~~~) calculations based on the worst-case FOCUS PEC~~~~sw~~ ~~and the acute RAC values for fish of the individual active substances (considering higher tier refinement options for the active substances) for application of BAS 736 00 F in ’winter cereals’~~**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Step 3 FOCUS scenario~~** | **~~Substance~~** | **~~Endpoint~~ ~~1)~~** | **~~AF~~** | **~~RAC~~*~~i~~* ~~[µg/L]~~** | **~~PEC~~*~~i~~* ~~[µg/L]~~ ~~2)~~** | **~~PEC~~*~~i~~* ~~/ RAC~~*~~i~~*** | **~~RQ~~~~mix~~** |
| ***~~fish, acute~~*** | | | | | | | |
| ~~R3 stream~~ | ~~Fluxapyroxad~~ | ~~SSD~~ ~~fish acute~~  ~~HC~~~~5~~ ~~= 148 µg/L~~ | ~~3~~ | ~~49.33~~ | ~~1.535~~ | ~~0.03~~ | ~~0.73~~ |
| ~~Azoxystrobin~~ | *~~O. mykiss,~~*  ~~LC~~~~50~~ ~~= 470 µg/L~~ | ~~100~~ | ~~4.7~~ | ~~3.111~~  ~~3.128~~ | ~~0.7~~ |
| ~~R4 stream~~ | ~~Fluxapyroxad~~ | ~~SSD~~ ~~fish acute~~  ~~HC~~~~5~~ ~~= 148 µg/L~~ | ~~3~~ | ~~49.33~~ | ~~0.845~~ | ~~0.02~~ | ~~0.62~~ |
| ~~Azoxystrobin~~ | *~~O. mykiss,~~*  ~~LC~~~~50~~ ~~= 470 µg/L~~ | ~~100~~ | ~~4.7~~ | ~~2.841~~ | ~~0.6~~ |

~~AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; RQ: risk quotient;~~

~~1)~~ ~~For details on the refined risk assessments and derivation of the resulting higher-tier RAC values for aquatic invertebrates see above.~~

**~~2)~~** ~~Only the worst-case PEC~~~~sw~~ ~~values out of the calculations for single and twofold application in winter cereals are used (for details on PEC~~~~sw~~ ~~calculations please refer to Part B, Section 9.8).~~

~~The RQ calculations based on acute higher tier data for fish indicate an acceptable risk for the application of BAS 736 00 F in ‘winter cereals’ with no need for additional mitigation measures.~~

**Table 9.5‑16: Aquatic organisms: acceptability of risk (PECmix/RAC < 1) for the formulation BAS 736 00 F for each organism group based on worst-case PECmix values following single and twofold application of BAS 736 00 F in ‘spring cereals’**

| **Group** |  | **Fish acute** | **Algae** | |
| --- | --- | --- | --- | --- |
| **Test species** |  | ***O. mykiss*** | ***P. subcapitata*** | |
| **Endpoint** |  | LC50 | ErC50 | |
| **(µg/L)** |  | 328.2 | 485.9 | |
| **AF** |  | 100 | 10 | |
| **RAC (µg a.s./L)** |  | 3.282 | 48.59 | |
| **FOCUS**  **Scenario** | **PEC mix-max (µg/L)#** | **PEC/RAC (= ETR)** | | |
| **Step 1** |  |  | |  |
|  | 105.213 | **32** | **2.2** | |
| **Step 2** |  |  | |  |
| N-Europe | 17.613 | **5.4** | 0.4 | |
| S-Europe | 32.479 | **9.9** | 0.7 | |
| **Step 3** |  |  | |  |
| D3 ditch | ~~1.585~~  1.584 | 0.5 | -- | |
| D4 pond | ~~1.265~~  1.271 | 0.4 | -- | |
| D4 stream | 1.502 | 0.5 | -- | |
| D5 pond | ~~0.495~~  0.497 | 0.2 | -- | |
| D5 stream | 1.337 | 0.4 | -- | |
| R4 stream | ~~3.922~~  2.666 | **~~1.2~~**  0.8 | -- | |

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in **bold**

# Worst-case PECs are derived from either single or twofold application in a risk envelope approach.For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 736 00 F in ‘spring cereals’ the calculated PECmix/RACPPP ratios indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and Step 1 - 3 PECsw mix values. ~~except for acute risk to fish at FOCUS stream scenario R4. Therefore, a refined risk assessment considering higher tier data for acute fish from fluxapyroxad is shown below (see Table 9.5‑17) in terms of using a risk quotient (RQ) approach for the critical stream scenario.~~

**~~Table 9.5‑17: Risk quotient (RQ~~~~mix~~~~) calculations based on the worst-case FOCUS PEC~~~~sw~~ ~~and the acute RAC values for fish of the individual active substances (considering higher tier refinement options for the active substances) for application of BAS 736 00 F in ’spring cereals’~~**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **~~Step 3 FOCUS scenario~~** | **~~Substance~~** | **~~Endpoint~~ ~~1)~~** | **~~AF~~** | **~~RAC~~*~~i~~* ~~[µg/L]~~** | **~~PEC~~*~~i~~* ~~[µg/L]~~** | **~~PEC~~*~~i~~* ~~/ RAC~~*~~i~~*** | **~~RQ~~~~mix~~** |
| ***~~fish, acute~~*** | | | | | | | |
| ~~R4 stream~~ | ~~Fluxapyroxad~~ | ~~SSD~~ ~~fish acute~~  ~~HC~~~~5~~ ~~= 148 µg/L~~ | ~~3~~ | ~~49.33~~ | ~~0.845~~ | ~~0.02~~ | ~~0.62~~ |
| ~~Azoxystrobin~~ | *~~O. mykiss,~~*  ~~LC~~~~50~~ ~~= 470 µg/L~~ | ~~100~~ | ~~4.7~~ | ~~2.841~~ | ~~0.6~~ |

~~AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; RQ: risk quotient;~~

~~1)~~ ~~For details on the refined risk assessments and derivation of the resulting higher-tier RAC values for aquatic invertebrates see above.~~

**~~2)~~** ~~Only the worst-case PEC~~~~sw~~ ~~values out of the calculations for single and twofold application in spring cereals are used (for details on PEC~~~~sw~~ ~~calculations please refer to Part B, Section 9.8).~~

~~The RQ calculations based on acute higher tier data for fish indicate an acceptable risk for the application of BAS 736 00 F in ‘spring cereals’ with no need for additional mitigation measures.~~

**Residue data in fish**

**Fluxapyroxad**

The log Pow of the active substance fluxapyroxad was determined to be 3.1 (BASF DocID 2007/1057001).

A BCF study with *L. macrochirus* (BASF DocID 2009/1012801) was conducted under flow-through conditions and resulted in a bioconcentration factor (BCF) of 86 and 93 for total radioactive residues (TRR) in whole fish for the 1 µg/L and the 10 µg/L dosing group, respectively. Bioconcentration factors calculated for the unchanged parent compound fluxapyroxad are 23 and 21 for edible tissue, 39 and 42 for inedible tissue, and 37 in whole fish of the low and high dose group, respectively. Rapid elimination of the test item was observed with a depuration half-life of < 1 day and a DT90 of 2.41 to 2.45 days. For details see EU dossier for fluxapyroxad, Document M-II, chapter 8.2.

Thus, the active substance fluxapyroxad has a low bioaccumulation potential and therefore, no accumulation in the food chain is to be expected.

**Azoxystrobin**

The logPow of the active substance azoxystrobin is 2.5 (EFSA Journal 2010; 8(4):1542), i.e. less than 3, therefore, no accumulation in the food chain is to be expected.

### Overall conclusions

**The standard and refined risk assessment for the fungicidal product BAS 736 00 F, the active substances fluxapyroxad and azoxystrobin as well as their major metabolites demonstrates that the application of BAS 736 00 F in ’winter and spring cereals’ according to good agricultural practice is of low risk to aquatic ecosystems.**

|  |
| --- |
| **Review Comments:**  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.  For active substances and relevant metabolites PECSW calculations were performed with FOCUS STEPS 1-2 (active substances and metabolites) and FOCUS STEP 3 - 4 (fluxapyroxad and azoxystrobin).  For azoxystrobin the mesocosms study was taken to consideration in the refined risk assessment.  For both active substances and their metabolites for the intended single and twofold application of BAS 736 00 F in cereals, the calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms without any mitigation measures.  Based on the mixure toxicity assessment, it can be concluded that the mitigation measures are required only for scenario R3 (10 m no spray buffer zone including a 10 m vegetated buffer strip). |

**References**

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**Appendix**

R script for the above-described comparison of model fit and calculation of the hazardous concentrations.

install.packages('ssdtools')

library(ssdtools)

library(ggplot2)

# read dataset

# this is the output of dput, which is used to create a data.frame from data entered in interactive spreadsheet

data <- structure(list(Concentration = c(212, 168, 572, 327, 370), Species = c("Oncorhynchus mykiss\n ", "Cyprinus carpio \n ", "Lepomis macrochirus \n ", "Promelas promelas \n ", "Cyprinodon variegatus"), Group = c(NA\_character\_, NA\_character\_, NA\_character\_, NA\_character\_, NA\_character\_)), row.names = c(NA, 5L), class = "data.frame")

# fix unacceptable column names

colnames(data) <- make.names(colnames(data))

# fit distributions

dist <- ssd\_fit\_dists(data, left = 'Concentration', dists = c('llogis', 'gamma', 'lnorm', 'lgumbel', 'weibull' 'burrIII3', 'gompertz))

# plot distributions

ssdtools::ssd\_plot\_cdf(dist)

# goodness of fit table

ssd\_gof(dist)

# save plot

# width and height are in inches, dpi (dots per inch) sets resolution

ggsave('fit\_dist\_plot.png', width = 8 , height = 6 , dpi = 300)

# select models to average

sel\_dists <- c('lnorm')

dist <- ssd\_fit\_dists(data, left = 'Concentration', dists = sel\_dists)

# plot model average

# to add confidence intervals set ci = TRUE in predict and ssd\_plot

# we recommend using nboot = 10000 in predict, although this may take several minutes to run

pred <- predict(dist, nboot = 10L)

ssd\_plot(data, pred, left = 'Concentration', label = 'Species', color = NULL, shape = NULL, hc = 5L, ci = FALSE,

shift\_x = 1.3, xlab = 'Concentration [mg/L]', ylab = 'Percent of Species Affected') +

ggtitle('') +

theme(panel.border = element\_blank(),

panel.grid.major = element\_blank(),

panel.grid.minor = element\_blank(),

panel.background = element\_rect(fill = NA, colour='black'),

axis.text = element\_text(color = 'black'),

legend.key = element\_rect(fill = NA, colour = NA)) +

expand\_limits(x = 572) +

scale\_color\_brewer(palette = 'Dark2', name = '-none-') +

scale\_shape(name = NULL)

# save plot

# width and height are in inches, dpi (dots per inch) sets resolution

ggsave('model\_average\_plot.png', width = 8 , height = 6 , dpi = 600)

# get confidence limits

# use the nboot argument in ssd\_hc to set the number of bootstrap samples

ssd\_hc(dist, percent = 5L, ci = TRUE, nboot = 1000L)

## Effects on bees (KCP 10.3.1)

### Toxicity data

Acute contact and oral toxicity studies on honey bees have been carried out with the active substance fluxapyroxad. Furthermore, a chronic toxicity study on adult honey bees, a toxicity study on honey bee larvae (repeated exposure) have been carried out, as well as an acute contact and oral toxicity studies on bumble bees with the active substance fluxapyroxad (partly tested as BAS 700 04 F).

Acute contact and oral toxicity studies on honey bees have been carried out with the active substance azoxystrobin. Furthermore, a chronic oral toxicity study on adult honey bees and an oral toxicity study on honeybee larvae have been carried out with azoxystrobin (tested as A12705B).

In addition to acute studies on honey bees, chronic toxicity studies on honey bee larvae and adults have been tested with BAS 736 00 F. All studies are listed in Table 9.6‑1, Table 9.6‑2 and Table 9.6‑3.

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

**Table 9.6‑1: Endpoints and effect values for fluxapyroxad relevant for the risk assessment for bees**

| **Species** | **Substance** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| *Apis mellifera* (adults) | fluxapyroxad | acute oral | LD50 (48 h) > 110.9 µg a.s./bee | EFSA Journal 2012;10(1):2522 2008/1010703 |
| *Apis mellifera* (adults) | fluxapyroxad | acute contact | LD50 (48 h) > 100 µg a.s./bee | EFSA Journal 2012;10(1):2522 2008/1010703 |
| *~~Apis mellifera~~* ~~(adults)~~ | ~~fluxapyroxad~~  ~~(tested as BAS 700 04 F)~~ ~~1)~~ | ~~chronic,~~  ~~10 d~~ | ~~LDD~~~~50~~ ~~(10 d) > 185 µg a.s./bee/day~~  ~~NOEDD (10 d) ≥ 185 µg a.s./bee/day~~ | ~~not EU evaluated~~  ~~2020/2083866~~ |
| *~~Apis mellifera~~* ~~(larvae)~~ | ~~fluxapyroxad~~ | ~~repeated exposure, 22 d~~ | ~~NOED (22 d) ≥ 80.0 µg a.s./larva ED~~~~50~~ ~~(22 d) > 80.0 µg a.s./larva~~ | ~~not EU evaluated~~  ~~2017/1036679~~ |
| *~~Bombus terrestris~~* ~~(adults)~~ | ~~fluxapyroxad (tested as BAS 700 04 F)~~ ~~1)~~ | ~~acute oral~~ | ~~LD~~~~50~~ ~~(96 h) > 176.3 µg a.s./bumble bee~~ | ~~not EU evaluated~~  ~~2014/1135445~~ |
| *~~Bombus terrestris~~* ~~(adults)~~ | ~~fluxapyroxad (tested as BAS 700 04 F)~~ ~~1)~~ | ~~acute contact~~ | ~~LD~~~~50~~ ~~(96 h) > 200.0 µg a.s./bumble bee~~ | ~~not EU evaluated~~  ~~2014/1135445~~ |

1) Study was conducted with the solo-formulation BAS 700 04 F containing 300.0 g fluxapyroxad/L.

**Table 9.6‑2: Endpoints and effect values for azoxystrobin relevant for the risk assessment for bees**

| **Species** | **Substance** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| *Apis mellifera* (adults) | azoxystrobin | acute oral | LD50 (48 h) > 25 µg a.s./bee | EFSA Journal 2010; 8(4):1542,  Jackson, D., Gough, J.B., Lewis, G.B., 1994, ICI5504/0859 |
| *Apis mellifera* (adults) | azoxystrobin | acute contact | LD50 (48 h) > 200 µg a.s./bee | EFSA Journal 2010; 8(4):1542,  Jackson, D., Gough, J.B., Lewis, G.B., 1994, ICI5504/0859 |
| *~~Apis mellifera~~*  ~~(adults)~~ | ~~azoxystrobin (tested as A12705B)~~ ~~1)~~ | ~~chronic, 10 d~~ | ~~LD~~~~50~~ ~~(10 d) = 17.41 µg a.s./bee/day~~  ~~NOED (10 d) = 10.01 µg a.s./bee/day~~ | ~~not EU evaluated~~  ~~Tänzler, V., 2015, A12705B\_13707~~ |
| *~~Apis mellifera~~*  ~~(larvae)~~ | ~~azoxystrobin (tested as A12705B)~~ ~~1)~~ | ~~single exposure~~ | ~~LD~~~~50~~ ~~(8 d) = 12.76 µg a.s./larva~~  ~~NOED (8 d) = 8.90 µg a.s./larva~~ | ~~not EU evaluated~~  ~~Ehmke, A., 2015,  A12705B\_13717~~ |

1) Used for conversion of µg A12705B/larva or /bee to µg a.s./larva or /bee. Content of a.s. nominal: 250 g/L. Content of a.s. analysed: 22.7% w/w corresponding to 248 g/L.

**Table 9.6‑3: Endpoints and effect values of BAS 736 00 F relevant for the risk assessment for bees**

| **Species** | **Product** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| *Apis mellifera* (adults) | BAS 736 00 F | acute oral | LD50 (48 h) > 432 µg/bee (corresponding to > 50.1 µg total a.s./bee) | not EU evaluated  2019/1061095 |
| *Apis mellifera* (adults) | BAS 736 00 F | acute contact | LD50 (72 h) > 1000 µg/bee (corresponding to > 116.0 µg total a.s./bee) | not EU evaluated  2019/1061095 |
| *Apis mellifera* (adults) | BAS 736 00 F | chronic, 10 d | LDD50 (10 d) = 36.6 µg/bee/day (corresponding to 4.2 µg total a.s./bee) 1)  NOEDD (10 d) = 19.2 µg/bee/day (corresponding to 2.2 µg total a.s./bee/day) 1) | not EU evaluated  2020/2080999 |
| *Apis mellifera* (larvae) | BAS 736 00 F | repeated exposure,  22 d | ED50 (22 d) = 188 µg/larva (corresponding to 21.8 µg total a.s./larva) 1)  NOED (22 d) = 120 µg/larva (corresponding to 13.9 µg total a.s./larva) 1) | not EU evaluated  2020/2081000 |

1) Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 736 00 F of 1.078 g/cm3.

* + - 1. **Justification for new endpoints**

Effects of the formulation BAS 736 00 F on honey bees were not evaluated as part of the EU assessment of the active substances fluxapyroxad or azoxystrobin. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

Repeated exposure studies on honey bee larvae and chronic oral honey bee studies, ~~both~~ with ~~fluxapyroxad (partly tested as BAS 700 04 F, containing 300 g fluxapyroxad/L) and~~ BAS 736 00 F, have been included to address the new data requirements according to Commission Regulation (EU) 1107/2009. ~~In addition, a single exposure honey bee larvae study and a chronic oral honey bee study with azoxystrobin (tested as A12705B, containing 250 g azoxystrobin/L), are submitted These studies provide further information on the toxicity of active substance to other honey bee life stages.~~

All chronic studies on bees which were previously not evaluated on EU level, were checked for their potential to calculate L/EC10/20 values in accordance with Commission Regulations (EU) 283/2013 and 284/2013, respectively. If a calculation was possible, the L/EC10/20 are provided in the corresponding study summary in Appendix 2. However, since these values are not relevant for the risk assessment, they are not listed in chapter 9.6.1.

### Risk assessment

The evaluation of the 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) and the EPPO 2010 risk assessment scheme (*OEPP/EPPO, 2010: Environmental risk assessment scheme for plant protection products, Chapter 10: Honey bees, PP 3/10 (3), Bulletin OEPP/EPPO Bulletin 40, 323–331*). The EFSA bee guidance document (EFSA Journal 2013; 11(7):3295) was not used as it has not been adopted by the Standing Committee on Plants, Animals, Food and Feed at the time of application.

The application of BAS 736 00 F is envisioned in cereals, a non-bee attractive crop. The following risk assessment is based on the worst-case maximum single application rate of 2.0 L BAS 736 00 F/ha (equivalent to 100 g fluxapyroxad/ha and 150 g azoxystrobin/ha; see Section 9 Chapter 9.1 for details).

#### Hazard quotients for bees

The risk to honey bees from the use of fluxapyroxad, azoxystrobin and BAS 736 00 F 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) (OEPP/EPPO, 2010: Chapter 10: Honey bees, PP 3/10 (3)) as follows.

A hazard quotient of less than 50 indicates a low risk to honey bees colonies in the field (see Table 9.6‑4, Table 9.6‑5 and Table 9.6‑6).

**Table 9.6‑4: First-tier assessment of the risk for bees due to the use of fluxapyroxad as contained in BAS 736 00 F** **according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | cereals | | |
| **Active substance** | fluxapyroxad | | |
| **Application rate (g a.s./ha)** | 2 x 100 | | |
| **Test design** | **LD50 (lab.) (µg a.s./bee)** | **Single application rate (g/ha)** | **QHO, QHC criterion: QH ≤ 50** |
| Oral toxicity | > 110.9 | 100 | < 0.9 |
| Contact toxicity | > 100 | < 1.0 |

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

**Table 9.6‑5: First-tier assessment of the risk for bees due to the use of azoxystrobin as contained in BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | cereals | | |
| **Active substance** | azoxystrobin | | |
| **Application rate (g a.s./ha)** | 2 x 150 | | |
| **Test design** | **LD50 (lab.) (µg a.s./bee)** | **Single application rate (g/ha)** | **QHO, QHC criterion: QH ≤ 50** |
| Oral toxicity | > 25 | 150 | < 6.0 |
| Contact toxicity | > 200 | < 0.8 |

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

**Table 9.6‑6**: **First-tier assessment of the risk for bees due to the use of BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | cereals | | |
| **Product** | BAS 736 00 F | | |
| **Application rate (L/ha)** | 2 x 2.0 | | |
| **Test design** | **LD50 (lab.) (µg/bee)** | **Single application rate (g/ha)** | **QHO, QHC criterion: QH ≤ 50** |
| Oral toxicity | > 432 | 2156 1) | < 5.0 |
| Contact toxicity | > 1000 | < 2.2 |

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 736 00 F of 1.078 g/cm3.

Commission Regulation (EU) No 284/2013 lists conditions under which testing of the formulated product is required. In accordance with the requirements set out in points 8.3.1 and 8.3.2 of Part A of the Annex to Regulation (EU) No 284/2013, formulated product testing is needed if the product contains more than one active substance and if the toxicity of a plant protection product cannot be reliably predicted to be either the same or lower than the toxicity of the active substances. For BAS 736 00 F, acute honey bee endpoints are available for all active substances and formulation. This data can be used to check the second condition, i.e. whether the formulated product shows unexpected toxicity.

The comparison of the acute endpoint obtained with the formulated product and the active substance endpoints, under consideration of the model deviation ratio (MDR), is shown in Table 9.6‑7. If the MDR is between 0.2 and 5, the observed and calculated mixture toxicities are considered in agreement. Comparing the acute toxicity of the active substances with the acute toxicity of the formulated product BAS 736 00 F, no indication for unpredicted product toxicity is given (MDR of 0.723 and 1.232 for acute oral and acute contact data, respectively).

**Table 9.6‑7: Measured acute toxicity of BAS 736 00 F and calculated mixture-toxicity comparison and presentation of the model deviation ratio (MDR)**

| **Test organisms  (Species)** | **Test type & endpoint** | **Measured toxicity of the a.s. [µg a.s./bee]** | | **Measured toxicity of BAS 736 00 F (LD50 PPP) [µg product/bee]** | **Calculated mixture toxicity (ECx mix-CA) [µg mixture/bee] 1)** | **MDR (ECx mix-CA / ECxPPP)** |
| --- | --- | --- | --- | --- | --- | --- |
|
| honey bee (*Apis mellifera*) | acute oral, 48 h LD50 | fluxapyroxad | > 110.9 | > 432 (> 50.1 µg total a.s./bee) | > 312.4 (> 36.2 µg total a.s./bee) | > 0.723 |
| azoxystrobin | > 25 |
| acute contact, 48 h LD50 | fluxapyroxad | > 100 | > 1000  (> 116.0 µg total a.s./bee) | > 1232.0 (> 142.9 µg total a.s./bee) | > 1.232 |
| azoxystrobin | > 200 |

PPP = Plant Protection Product; CA = concentration addition; MDR = model deviation ratio

1) The theoretical formulation toxicity of the product was re-calculated based on the measured toxicity data of the active substances and their nominal content within the formulation (i.e. 50 g fluxapyroxad/L and 75 g azoxystrobin/L) and a product density of 1.078 g/cm3 from the study.

Under Regulation (EC) No 1107/2009, no adopted risk assessment scheme currently exists for chronic honey bee or honey bee larvae studies. ~~Nevertheless, additional studies were carried out with fluxapyroxad (BAS 700 F), azoxystrobin (BAS 9164 F) and BAS 736 00 F. For fluxapyroxad, chronic toxicity study on honey bees resulted in a NOED ≥ 185 µg/bee/day. The NOED derived from the repeated exposure study on honey bee larvae resulted in a NOED of ≥ 80.0 µg/larva.~~ ~~For azoxystrobin, chronic toxicity study on honey bees resulted in a NOED 10.01 µg/bee/day. The NOED derived from the single exposure study on honey bee larvae resulted in a NOED of 8.90 µg/larva.~~ For BAS 736 00 F, the chronic toxicity study on honey bees resulted in a NOEDD of 2.2 µg total a.s./bee/day. The NOED derived from the repeated exposure study on honey bee larvae resulted in a NOED of 13.9 µg a.s./larva. In the absence of clear guidance (noted and agreed by member states) a preliminary risk assessment according to the current legal requirements (SANCO/10329/2002 and EPPO 2010) has been conducted and is presented below.

Repeated exposure of adult honey bees and immature life stages within the hive is realistic for active substances but not for the formulated product (formulants have different physical and chemical properties). Conclusively, chronic exposure of adult bees of more than 10 days and repeated exposure of honey bee larvae to the product as a whole is extremely unrealistic. Hence, data of BAS 736 00 F is considered a worst-case scenario and sufficiently covers exposure to the product and to the active substances. In a worst-case approach, the following risk assessment will based on data for BAS 736 00.

For the **chronic risk assessment for adult honey bees and honey bee larvae**, the revised EPPO scheme (2010) suggests calculating the ratio between the NOEL (oral) and the exposure. This approach has been originally proposed for seed treatments but can be directly applied to foliar applications as well. For adult bees, the exposure is assessed through the amount of residues that may be ingested by a bee in one day. The ratio between the NOEL (= NOED in µg a.s./bee/day) and the exposure (also in µg a.s./bee/day) is then calculated as follows:

For the risk assessment the exposure of larvae is estimated as the amount of residues that may be ingested by the larvae during their complete larval stage (feeding period of five days) as a worst case assumption. For larvae, the ratio between the NOEL (in µg a.s./larva) and the exposure (residues ingested over the five-day feeding period in µg a.s./larva) is calculated by the following equation:

Following EPPO (2010) the expected worst-case residue consumption of larvae and adult bees was calculated. For fluxapyroxad and azoxystrobin, no specific RUD residue values were reported. Therefore, overall RUD residue values for spray applications have been used for exposure estimation as reported in EFSA (2017). In order to be protective, we suggest using the 3rd Quantile data which are well above the more realistic median values. Expected residues in nectar and pollen are calculated using the maximum single application of BAS 736 00 F (100 g fluxapyroxad/ha and 150 g azoxystrobin/ha; see Table 9.6‑8).

**Table 9.6‑8:Residue values of the active substances in pollen and nectar**

|  |  |  |
| --- | --- | --- |
|  | **3rd quartile RUD** | **Expected residues based on proposed GAP** |
| **Pollen** | | |
| (total) a.s. in BAS 736 00 F  (Application rate 100 g fluxapyroxad/ha and 150 g azoxystrobin/ha | 127.4 mg a.s./kg 1) | 15.93 mg a.s./kg |
| **Nectar** | | |
| (total) a.s. in BAS 736 00 F  (Application rate 100 g fluxapyroxad/ha and 150 g azoxystrobin/ha | 7.98 mg a.s./kg 1) | 1.00 mg a.s./kg |

1) Overall RUD values from EFSA supporting publication on residues in bee relevant matrices (EFSA 2017).

To calculate the expected consumption of the relevant matrixes EPPO 2010 refers to a review by Rortais *et al. (2005).* For adult honey bees, only nectar consumption is relevant as adult bees do not consume pollen.

In Rortais *et al. (2005)* the maximum amount of sugar an adult bee consumes per day is given as 128 mg/bee/day. Based on nectar sugar concentration of 30% this corresponds to a total consumption of approximately 426.7 mg/bee/day, which can be considered an unrealistic worst-case scenario. In the absence of clear guidance, the nectar sugar concentration was taken from Rortais *et al*. (2005),which cite a range of sugar concentrations in nectars between 5-80% specifically mentioning 40% as representative in bee attractive crops. This range suggests that 30% sugar concentration can be considered conservative for crop plants, which is well supported by the literature (Pamminger *et al*. 2019). For honey bee larvae Rortais *et al.* (2005) gives a maximum of 59.4 mg sugar/5days, which corresponds to a nectar consumption of 198 mg/5days based on 30% sugar concentration in nectar. In addition to their nectar requirements honey bee larvae consume up to 2 mg pollen/5days (Babendreier *et al*. 2004). It is to be noted that the pollen consumption values mentioned in Rortais *et al.* (2005) based on a citation of Babendreier *et al*. (2004) are not the values which are mentioned in the original publication Babendreier *et al*. (2004).

To calculate the residue intake of BAS 736 00 F by adult honey bees and honey bee larvae, the consumed amounts of pollen and nectar are multiplied with relevant measured residue in nectar and pollen after application of BAS 736 00 F (see Table 9.6‑9). The calculated chronic TER values are given in Table 9.6‑10. These TERs are compared to the trigger of 1 as proposed in the revised EPPO scheme (2010). **Given the protective worst-case assumptions underlying this risk assessment (detailed above), as well as the fact that all calculated TERs far exceed the suggested trigger by at least a factor of 5, it can be concluded that the risk for chronic adult and developmental exposure to honey bees can be considered acceptable.**

**Table 9.6‑9: Total residue intake for adult honey bees and larvae following exposure to BAS 736 00 F according to the proposed uses**

|  |  |  |
| --- | --- | --- |
| **Honey bee stage** | **Adult** | **Larva (over 5 days)** |
| Residue in pollen | 15.93 mg total a.s./kg  (= 0.01593 µg total a.s./mg) | 15.93 mg total a.s./kg  (= 0.01593 µg total a.s./mg) |
| Pollen consumption | 0 | 2.0 mg/larva |
| Residue intake through pollen | 0 µg total a.s./bee/day | 0.032 µg a.s./larva |
| Residue in nectar | 1.00 mg total a.s./kg  (= 0.001 µg total a.s./mg) | 1.00 mg total a.s./kg  (= 0.001 µg total a.s./mg) |
| Nectar consumption | 426.7 mg/bee/day | 198.0 mg/larva |
| Residue intake through nectar | 0.426 µg a.s./bee/day | 0.198 µg a.s./larva |
| **Total residue intake** | **0.426 µg a.s./bee/day** | **0.229 µg a.s./larva** |

**Table 9.6‑10: Chronic risk to adult bees and larvae following the use of BAS 736 00 F according to the proposed use pattern using the TER approach**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Honey bee stage** | **Exposure route** | **Endpoint** | **Worst case residue intake** | **TERch** | **Trigger value** |
| Adult | Oral | 2.2 µg total a.s./bee/day | 0.43µg a.s./bee/day | 5.1 | 1 |
| Larvae | Oral | 13.9 µg total a.s./larva | 0.23µg a.s./larva | 60.4 | 1 |

The underlying assumptions of the revised EPPO (2010) risk assessment for chronic adult bees and honey bee larvae largely comply with the proposals presented in the EFSA bee guidance document:

* in both approaches the chronic adult and larvae endpoints are set into relation to exposure which is based on pollen and nectar consumption.
* in both approaches the assumed amount of pollen and nectar consumption and the relevant time-frame is identical as it is based on the same literature references.
* the RUD values used from the EFSA supporting publication 2017 are based on a review and quality evaluation of available residue studies. The request of EFSA for the supporting publication was the limited availability of residue data at the time of the finalization of the EFSA bee guidance document. The EFSA supporting publication 2017 reflects therefore the current knowledge status.
* the possibility to refine exposure by using a time-weighted-average factor is a common refinement option for risk assessment of non-target organisms which is also mentioned in the EFSA bee guidance document.

However, in some respects this proposal deviates from the EFSA bee guidance document. Main differences lie in the endpoints and triggers used. In the EFSA bee guidance document it is proposed to use the LDD50 endpoint for chronic adult and the NOED for honey bee larvae. At the same time the proposed chronic adult trigger in the EFSA bee guidance document for the ETR (exposure toxicity ratio) based on LDD50 is 0.03, corresponding to a TER trigger (toxicity exposure ratio) of 33.3. The ETR trigger for the larvae risk assessment in the EFSA bee guidance document is 0.2 (corresponding to a TER trigger of 5). In EPPO 2010 the proposed TER trigger is 1 based on NOED endpoint for the chronic adult and the larvae risk assessment.

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

Not relevant.

### Effects on bumble bees

For bumble bees no specific data requirement exists under regulation (EC) No 1107/2009. ~~Nevertheless, to support the application an acute oral and contact study was conducted with the active substance fluxapyroxad (tested as BAS 700 04 F). The oral and contact LD~~~~50~~ ~~were determined to be > 176.3 µg a.s./bumble bee and > 200.0 µg a.s./bumble bee, respectively. Both endpoints exceed (are in the same range as) the acute endpoints for honey bees suggesting that fluxapyroxad as contained in BAS 736 00 F poses no unacceptable risk to bumble bees at the proposed use rate.~~

### Effects on solitary bees

No reliable and validated testing methods for solitary bees are currently available and no specific data requirement exists under regulation (EC) No 1107/2009. The EFSA bee guidance document (EFSA Journal 2013; 11(7):3295) has not been adopted at the time of application. Therefore, no studies with solitary bees have been performed.

### Overall conclusions

**The hazard quotients for BAS 736 00 F and the active substances fluxapyroxad and azoxystrobin for acute oral and acute contact exposure of honey bees are considerably below the Commission Regulation (EU) 546/2011 trigger value of 50. Based on the available information it can be concluded that no unacceptable risk to honey bees is expected from applications of BAS 736 00 F according to the proposed uses.** **This is confirmed by a risk assessment following EPPO (2010) for chronic exposure to adult honey bees and repeated exposure to honey bee larvae.**

|  |
| --- |
| **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 736 00 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.  There is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of EPPO 2010 approach at national level. |

References

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Pamminger, T., Becker, R., Himmelreich, S., Schneider, C. W., & Bergtold, M. (2019). The nectar report: quantitative review of nectar sugar concentrations offered by bee visited flowers in agricultural and non-agricultural landscapes. *PeerJ*, *7*, e6329.

Rortais, A., Arnold, G., Halm, M. P., & Touffet-Briens, F. (2005). Modes of honey bees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie, 36(1), 71-83.

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

### Toxicity data

The toxicity of BAS 736 00 F to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri* and Tier II tests on *T. pyri*, *A. rhopalosiphi* and *Chrysoperla carnea.* All studies are listed in Table 9.7‑1. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

**Table 9.7‑1: Endpoints and effect values for BAS 736 00 F relevant for the risk assessment for non-target arthropods**

| **Species** | **Product** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| *Typhlodromus pyri* (protonymphs) | BAS 736 00 F | laboratory test glass plates 2D exposure | LR50 = 2.75 L/ha  Corrected mortality:  -1.0% at 0.25 L/ha 0% at 0.5 L/ha 1.0% at 1.0 L/ha 8.2% at 2.0 L/ha 100% at 4.0 L/ha | not EU evaluated  2019/1061102 |
| *Aphidius rhopalosiphi* (adults) | BAS 736 00 F | laboratory test glass plates 2D exposure | LR50 = 2.67 L/ha  Corrected mortality:  0% at 0.25 L/ha 0% at 0.5 L/ha 7.9% at 1.0 L/ha 18.4% at 2.0 L/ha 92.1% at 4.0 L/ha | not EU evaluated 2019/1061100 |
| *Typhlodromus pyri* (protonymphs) | BAS 736 00 F | extended laboratory test bean leaf discs, 2D exposure | LR50 > 4.0 L/ha ER50 > 4.0 L/ha  Corrected Mortality: 1)  0% at 0.25 L/ha 0% at 0.5 L/ha -1.0% at 1.0 L/ha -1.0% at 2.0 L/ha 1.0% at 4.0 L/ha  Effects on reproduction: 1)  4.4% at 0.25 L/ha 0.9% at 0.5 L/ha 3.6% at 1.0 L/ha -3.8% at 2.0 L/ha 7.8% at 4.0 L/ha | not EU evaluated 2019/1061104 |
| *Aphidius rhopalosiphi* (adults) | BAS 736 00 F | extended laboratory test barley plants, 3D exposure | LR50 > 4.0 L/ha ER50 > 4.0 L/ha  Corrected Mortality: 1)  0% at 0.25 L/ha -3.6% at 0.5 L/ha 0% at 1.0 L/ha 3.6% at 2.0 L/ha 0% at 4.0 L/ha  Effects on reproduction: 1)  0% at 0.25 L/ha -7.9% at 0.5 L/ha 6.9% at 1.0 L/ha 4.6% at 2.0 L/ha -2.8% at 4.0 L/ha | not EU evaluated 2019/1061101 |
| *Chrysoperla carnea* (larvae) | BAS 736 00 F | extended laboratory test bean leaves, 2D exposure | LR50 > 4 L/ha  ER50 > 4 L/ha  Corrected Mortality: 1)  2.1% at 0.25 L/ha 0% at 0.5 L/ha 12.8% at 1.0 L/ha 14.9% at 2.0 L/ha 46.8% at 4.0 L/ha  Hatching rate: 74.4% at 0.25 L/ha 73.9% at 0.5 L/ha 74.2% at 1.0 L/ha 73.9% at 2.0 L/ha 74.1% at 4.0 L/ha | not EU evaluated 2019/2054735 |

1) Positive values indicate a decrease in survival or reproduction; negative values indicate an increase in survival or reproduction, compared to the control.

#### Justification for new endpoints

Effects of BAS 736 00 F on non-target arthropods other than bees were not evaluated as part of the EU assessment of the active substances fluxapyroxad and azoxystrobin. 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 approach recommended in the ESCORT 2 guidance document, ESCORT 3, and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002)*.*

#### Risk assessment for in-field exposure

The application of BAS 736 00 F is envisioned in cereals. The following risk assessment is based on the worst-case field application rate of 2 × 2.0 L/ha (see Section 9 Chapter 9.1 for details).

The in-field exposure (Predicted Environmental Rate, PER) is calculated according to the ESCORT 2 Guidance Document using the following equation:

Default foliar and soil MAF values following multiple applications are given in the ESCORT 2 Guidance Document and are the following for BAS 736 00 F and its application scheme.

MAF (leaf substrate) = 1.7

MAF (soil) = 1.9

As a pre-emergence or early post-emergence application is not intended for the use of BAS 736 00 F (see Section 9 Chapter 9.1 for details), the MAF (soil) will not be considered in the following risk assessment. Thus, the PERin-field is 3.4 L/ha.

The potential risk for non-target arthropods exposed in-field to BAS 736 00 F was assessed by calculating the hazard quotient (HQ = exposure/toxicity, see Table 9.7‑2) for tier I standard laboratory studies according to the formula:

For higher tier laboratory studies risk is acceptable if the PERin-field is below the relevant endpoint (see Table 9.7‑2).

**Table 9.7‑2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | cereals | | |
| **Product** | BAS 736 00 F | | |
| **Application rate (L/ha)** | 2 x 2.0 | | |
| **MAF** | 1.7 (vegetation) | | |
| **Test species** | **Tier I** | | |
| **LR50 (lab.) [L/ha]** | **PERin‑field [L/ha]** | **HQin-field criterion: HQ ≤ 2** |
| *Typhlodromus pyri* | > 2.75 | 3.4 | < 1.2 |
| *Aphidius rhopalosiphi* | > 2.67 | < 1.3 |
| **Test species** | **Higher-tier** | | |
| **Endpoint [L/ha]** | **PERin‑field [L/ha]** | **PERin-field below rate with ≤ 50% effect?** |
| *Typhlodromus pyri* | LR50 > 4.0  ER50 > 4.0 | 3.4 | yes yes |
| *Aphidius rhopalosiphi* | LR50 > 4.0  ER50 > 4.0 | yes yes |
| *Chrysoperla carnea* | LR50 > 4.0  ER50 > 4.0 | yes yes |

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

#### Risk assessment for off-field exposure

Exposure of non-target arthropods living in off-field areas to BAS 736 00 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 from in-field foliar PER values in conjunction with drift values listed in Appendix IV of the ESCORT 2 guidance document:

A vegetation distribution or dilution factor is included in the equation when calculating PER values from toxicity endpoints derived from two-dimensional studies (Table 9.7‑3). A dilution factor of 10 is recommended by ESCORT 2.

For 2 applications of BAS 736 00 F in cereals, the drift value at 1 m distance is 2.38% of the application rate (82nd percentile drift). The drift factor (% drift/100) is therefore 2.38/100 = 0.0238.

**Table 9.7‑3: PERoff-field values following application of BAS 736 00 F**

| **Study type [Exposure scenario]** | **Maximum PERin-field [L/ha]** | **Drift factor [% drift/100]** | **Vegetation distribution factor** | **PERoff-field [L/ha]** |
| --- | --- | --- | --- | --- |
| 2D | 3.4 | 0.0238 | 10 / 5 | 0.008 / 0.0162 |
| 3D | -- | 0.08 |

To assess the potential risk of BAS 736 00 F to off-field non-target arthropods (see Table 9.7‑4), the PERoff-field (Table 9.7‑3) is compared to the toxicity endpoints of tier I standard laboratory studies according to the following equation:

For higher tier laboratory studies risk is acceptable if the PERoff-field is below the relevant endpoint.

ESCORT 2 recommends a correction factor of 10 for Tier I and 5 for higher Tier data in the off-field risk assessment to account for extrapolation from testing just few representative species to the species diversity expected in off-field areas.

**Table 9.7‑4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Intended use** | cereals | | | | |
| **Product** | BAS 736 00 F | | | | |
| **Application rate (L/ha)** | 2 x 2.0 | | | | |
| **MAF** | 1.7 (vegetation) | | | | |
| **vdf** | 10 or 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* | > 2.75 | 2.38 | 0.008 | 10 | < 0.03 |
| *Aphidius rhopalosiphi* | > 2.67 | < 0.03 |
| **Test species** | **Higher-tier** | | | | |
| **Endpoint [L/ha]** | **Drift rate**  **(%)** | **PERoff‑field [L/ha]** | **CF 1)** | **corr. PERoff-field below rate with ≤ 50% effect?** |
| *Typhlodromus pyri* | LR50 > 4.0  ER50 > 4.0 | 2.38 | 0.008/ 0.0162 | 5 | yes  yes |
| *Chrysoperla carnea* | LR50 > 4.0  ER50 > 4.0 |
| *Aphidius rhopalosiphi* | LR50 > 4.0  ER50 > 4.0 | 0.08 | yes  yes |

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

1) Standard 5-fold uncertainty (correction) factor should be included to the calculation to cover the inter-species variability in sensitivity of off-field non-target arthropod species. However, as additional species are tested, the uncertainty is reduced and no additional safety factor was applied.

#### Additional higher-tier risk assessment

Not relevant.

#### Risk mitigation measures

No risk mitigation needed.

### Overall conclusions

**Based on the results of the conducted first and higher tier risk assessments it can be concluded that low risk for non-target arthropods is expected from the use of BAS 736 00 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:**  Based on the results of the conducted risk assessment it can be concluded that low risk for non-target arthropods is expected from the use of BAS 736 00 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats. |

## 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) have been carried out with fluxapyroxad, azoxystrobin and relevant metabolites. Full details of these studies are provided in the respective EU documents. Additionally, new toxicity studies on earthworms and other non-target meso- and macrofauna have been conducted ~~with one or more of the active ingredients and their relevant metabolites.~~ All studies are listed in Table 9.8‑1, Table 9.8‑2 and Table 9.8‑3.

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

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

| **Species** | **Substance/metabolite** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| **Acute #** | | | | |
| *Eisenia fetida* | fluxapyroxad | Mixed into substrate 14 d acute 5% peat | LC50 > 1000 mg/kg dry soil | EFSA Journal 2012; 10(1):2522  2009/1072245 |
| *Eisenia fetida* | Metabolite, Reg. No. 5 069 089 M700F001 | Mixed into substrate 14 d acute 10% peat | LC50 > 1000 mg/kg dry soil | EFSA Journal 2012; 10(1):2522  2009/1050216 |
| *Eisenia fetida* | Metabolite, Reg. No. 5 435 595 M700F002 | Mixed into substrate 14 d acute 10% peat | LC50 > 1000 mg/kg dry soil | EFSA Journal 2012; 10(1):2522  2009/1072244 |
| **Chronic** | | | | |
| *~~Eisenia fetida~~* | ~~fluxapyroxad~~ | ~~Mixed into substrate 56 d chronic 10% peat~~ | ~~NOEC = 122 mg/kg dry soil~~ **~~NOEC~~~~CORR~~ ~~= 61 mg/kg dry soil~~ ~~\*~~** | ~~not EU evaluated~~  ~~2014/1135449~~ |
| *Eisenia fetida* | Metabolite, Reg. No. 5 069 089 M700F001 | Mixed into substrate 56 d chronic 5% peat | **NOEC ≥ 5.33 mg/kg dry soil** | EFSA Journal 2012; 10(1):2522  2008/1033932 |
| *Eisenia fetida* | Metabolite, Reg. No. 5 435 595 M700F002 | Mixed into substrate 56 d chronic 5% peat | **NOEC ≥ 2.56 mg/kg dry soil ~~1)~~** | EFSA Journal 2012; 10(1):2522  2008/1017010 |
| *~~Folsomia candida~~* | ~~fluxapyroxad~~ | ~~Mixed into substrate 28 d chronic 5% peat~~ | **~~NOEC ≥ 500 mg/kg dry soil~~** ~~EC~~~~10~~ ~~> 500 mg/kg dry soil~~  ~~NOEC~~~~CORR~~ ~~≥ 250 mg/kg dry soil~~ ~~\*~~ ~~EC~~~~10~~~~CORR~~ ~~> 250 mg/kg dry soil~~ ~~\*~~ | ~~not EU evaluated~~  ~~2014/1135433~~ |
| *Folsomia candida* | Metabolite, Reg. No. 5 435 595 M700F002 | Mixed into substrate 28 d chronic 5% peat | **NOEC ≥ 1000 mg/kg dry soil** | EFSA Journal 2012; 10(1):2522  2009/1045472 |
| *~~Hypoaspis aculeifer~~* | ~~fluxapyroxad~~ | ~~Mixed into substrate 14 d 5% peat content~~ | **~~NOEC ≥ 1000 mg/kg dry soil~~** ~~EC~~~~10~~ ~~> 1000 mg/kg dry soil~~  ~~NOEC~~~~CORR~~ ~~≥ 500 mg/kg dry soil~~ ~~\*~~ ~~EC~~~~10~~~~CORR~~ ~~> 500 mg/kg dry soil~~ ~~\*~~ | ~~not EU evaluated 2016/1122885~~ |

Values shown in **bold** are relevant for the conclusion of the risk assessment.

# Acute studies listed for reference only but not used in the risk assessment according to Commission Regulation (EU) 283/2013.

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 due to al log POW > 2 of fluxapyroxad.

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

| **Species** | **Substance/metabolite** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| **Acute #** | | | | |
| *Eisenia fetida* | azoxystrobin | Mixed into substrate 14 d 10% peat content | LC50 = 283 mg/kg dry soil  **LC50 CORR= 142 mg/kg dry soil** \* | EFSA Journal 2010; 8(4):1542,  Coulson J.M., Yearsdon H.A., Flemming T.M., ICI5504/0904 |
| *Eisenia fetida* | A12705H 1)  (250 SC) | Mixed into substrate 14 d 10% peat content | LC50 = 881 mg/kg dry soil | EFSA Journal 2010; 8(4):1542,  Bembridge J.D., Jackson, D., Coulson, J.M., ICI5504/0905 |
| *Eisenia fetida* | Metabolite,  R234886 | Mixed into substrate 14 d 10% peat content | **LC50 > 1000 mg/kg dry soil** | EFSA Journal 2010; 8(4):1542,  Friedrich, S., 2002, R234886/0001 |
| *Eisenia fetida* | Metabolite,  R402173 | Mixed into substrate 14 d 10% peat content | **LC50 > 1000 mg/kg dry soil** | EFSA Journal 2010; 8(4):1542,  Friedrich, S., 2008a, SYN501114/0001 |
| *Eisenia fetida* | Metabolite,  R401553 | Mixed into substrate 14 d 10% peat content | **LC50 > 1000 mg/kg dry soil** | EFSA Journal 2010; 8(4):1542,  Friedrich, S., 2008, SYN501657/0006 |
| **Chronic** | | | | |
| *Eisenia fetida* | azoxystrobin  (tested as A12705B) | Mixed into substrate 56 d 10% peat content | NOEC = 20 mg a.s./kg dry soil  **NOEC CORR = 10 mg a.s./kg dry soil** \* | EFSA Journal 2010; 8(4):1542,  Roembke, J., Moser, T., 2000, ICI5504/0903 |
| *~~Eisenia fetida~~* | ~~Metabolite,~~  ~~R234886~~ | ~~Mixed into substrate 56 d 5% peat content~~ | ~~NOEC = 16 mg/kg dry soil~~ | ~~not EU evaluated~~  ~~Friedrich, 2010a~~  ~~R234886\_10001~~ |
| *Folsomia candida* | azoxystrobin  (tested as A12705B) | Mixed into substrate 28 d 5% peat content | **NOEC = 50 mg a.s./kg dry soil**  NOEC CORR = 25 mg a.s./kg dry soil \* | EFSA Journal 2010; 8(4):1542,  Barth, M., 2001, ICI5504/1319 |
| *~~Hypoaspis aculeifer~~* | ~~azoxystrobin~~  ~~(tested as A12705B)~~ | ~~Mixed into substrate 14 d 5% peat content~~ | **~~NOEC = 1000 mg a.s./kg dry soil~~**  ~~NOEC~~ ~~CORR~~ ~~= 500 mg a.s./kg dry soil~~ ~~\*~~ | ~~not EU evaluated~~  ~~Schulz, L., 2017; A12705B\_13887~~ |

Values shown in **bold** are relevant for the conclusion of the risk assessment.

# Acute studies listed for reference only but not used in the risk assessment according to Commission Regulation (EU) 283/2013.

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 due to the log Pow > 2 of azoxystrobin.

1) A12705B and A12705H contain the same active ingredient content and a nearly identical co-formulant profile except a marginal decrease for one co-formulants and marginal increases in two further co-formulants within A12705B compared to A12705H.

**Table 9.8‑3: Endpoints and effect values of BAS 736 00 F relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Product** | **Exposure System** | **Results** | **Reference** |
| **Chronic** | | | | |
| *Eisenia fetida* | BAS 736 00 F | Mixed into substrate  56 d 10% peat content | NOEC = 284.0 mg/kg dry soil (equivalent to 13.2 mg fluxapyroxad and 19.8 mg azoxystrobin/kg dry soil) EC10 = 295.9 mg/kg dry soil (equivalent to 13.7 mg fluxapyroxad and 20.6 mg azoxystrobin/kg dry soil)  NOECCORR = 16.5 mg total a.s./kg dry soil 1) \* **EC10 CORR = 17.2 mg total a.s./kg dry soil 1) \*** | not EU evaluated 2019/1061096 |
| *Folsomia candida* | BAS 736 00 F | Mixed into substrate  28 d 5% peat content | NOEC = 147 mg/kg dry soil (equivalent to 6.8 mg fluxapyroxad and 10.2 mg azoxystrobin/kg dry soil) **EC10 = 232 mg/kg dry soil (equivalent to 10.8 mg fluxapyroxad and 16.1 mg azoxystrobin/kg dry soil)**  NOECCORR = 8.5 mg total a.s./kg dry soil 1) \* EC10 CORR = 13.5 mg total a.s./kg dry soil 1) \* | not EU evaluated 2019/1061097 |
| *Hypoaspis aculeifer* | BAS 736 00 F | Mixed into substrate  14 d 5% peat content | NOEC = 190.7 mg/kg dry soil (equivalent to 8.8 mg fluxapyroxad and 13.3 mg azoxystrobin/kg dry soil) **EC10 = 438.9 mg/kg dry soil (equivalent to 20.4 mg fluxapyroxad and 30.5 mg azoxystrobin/kg dry soil)**  NOECCORR = 11.1 mg total a.s./kg dry soil 1) \* EC10 CORR = 25.4 mg total a.s./kg dry soil 1) \* | not EU evaluated 2019/1061098 |

Values shown in **bold** are relevant for the conclusion of the risk assessment.

\* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 due to the log Pow > 2 of fluxapyroxad and azoxystrobin.

1) Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 736 00 F of 1.078 g/cm3.

#### Justification for new endpoints

Effects of the formulation BAS 736 00 F on earthworms and other non-target soil organisms (meso- and macrofauna) were not evaluated as part of the EU assessment of the active substances fluxapyroxad or azoxystrobin. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

~~New chronic studies testing the effects of fluxapyroxad on earthworms, collembolans and soil mites which were not evaluated as part of the EU assessment of the active substance fluxapyroxad. As they provide valuable additional information they have been included in the evaluation. This applies to the new soil mites chronic study of azoxystrobin and earthworm study of its metabolite.~~

All chronic studies on earthworms and collembolans after guidelines OECD 222 and OECD 232 respectively, were checked for their potential to calculate EC10/20 values. If a calculation was possible, the EC10/20 are provided in the corresponding study summary in Appendix 2 and the EC10 is listed in Chapter 9.8.1.

For the risk assessment, both NOEC and EC10 (if available) are used. The conclusion, however, will be based on the EC10 if reliable. If the EC10 is not reliable or could not be calculated the NOEC is considered the relevant endpoint for 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).

For substances with a log Pow > 2 an endpoint correction by a soil factor of 2 (foc) must be considered. The log Pow values of the fluxapyroxad metabolites M700F001 and M700F002 and the azoxystrobin metabolites R234886, R402173 and R401553 are < 2. Therefore, the endpoints are not corrected. The endpoints of the active substances fluxapyroxad and azoxystrobin were corrected (10% peat content), due to a log Pow > 2. The EPPO earthworm scheme 2002 recommends an endpoint correction for earthworm studies with 10% peat content only. According to EPPO there is no need to correct endpoints derived from studies conducted with 5% peat. It should be noted that EFSA proposed an endpoint correction for 5% peat studies in its technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA supporting publication 2015: EN 924. 62 pp.). However, this correction is not justified by specific data and is not adopted by all member states. Therefore, corrected and uncorrected values are given in the following risk assessment and the conclusion is based on endpoints which are in line with the EPPO earthworm scheme 2002.

#### First-tier risk assessment

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), Chapter 8.7.2. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for the relevant metabolites R234886, R402173 and R401553 of azoxystrobin. In contrast, multi-annual accumulation needs to be considered for the active substance azoxystrobin and fluxapyroxad as well as for the fluxapyroxad metabolites M700F001 and M700F002.

The potential risk of BAS 736 00 F, fluxapyroxad, azoxystrobin and their relevant metabolites to earthworms and other non-target soil macro-organisms was assessed by comparing the maximum PECsoil values with NOEC or EC10 values, to generate long-term TER values (TERlt, Table 9.8‑4 to Table 9.8‑6).

The TER was calculated as follows:

**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 use of fluxapyroxad as contained in BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | 2 x 100 g fluxapyroxad/ha in cereals | | |
| **Chronic effects on earthworms** | | | |
| **Active substance/metabolite** | **Endpoint  (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| ~~fluxapyroxad~~ | ~~NOEC~~~~= 122~~ **~~NOEC~~~~CORR~~ ~~= 61~~** | ~~0.078~~ ~~\*~~ | ~~1564~~  **~~782~~** |
| Metabolite, Reg. No. 5 069 089 M700F001 | **NOEC ≥ 5.33** | 0.004 \* | **≥ 1333** |
| Metabolite, Reg. No. 5 435 595 M700F002 | **NOEC ≥ 2.56** | 0.023 \* | **≥ 111** |
| **Chronic effects on other soil meso- and macrofauna** | | | |
| **Collembola (*Folsomia candida*)** | | | |
| **Active substance/metabolite** | **Endpoints (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| ~~fluxapyroxad~~ | **~~NOEC ≥ 500~~** ~~EC~~~~10~~ ~~> 500~~  ~~NOEC~~~~CORR~~ ~~≥ 250  EC~~~~10~~~~CORR~~ ~~> 250~~ | ~~0.078~~ ~~\*~~ | **~~≥ 6410~~**  ~~> 6410~~  ~~≥ 3205~~  ~~> 3205~~ |
| Metabolite, Reg. No. 5 435 595 M700F002 | **NOEC ≥ 1000** | 0.023 \* | **≥ 43478** |
| **Soil mites (*Hypoaspis aculeifer*)** | | | |
| ~~fluxapyroxad~~ | **~~NOEC ≥ 1000~~** ~~EC~~~~10~~ ~~> 1000 NOEC~~~~CORR~~ ~~≥ 500~~  ~~EC~~~~10~~~~CORR~~ ~~> 500~~ | ~~0.078~~ ~~\*~~ | **~~≥ 12821~~** ~~> 12821 ≥ 6410 > 6410~~ |

Values in **bold** are relevant for the conclusion of the risk assessment.

\* PECsoil, accu. For details please refer to section 8, chapter 8.7.

**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 azoxystrobin as contained in BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | 2 x 150 g azoxystrobin/ha in cereals | | |
| **~~Acute effects on earthworms~~** | | | |
| **~~Active substance/metabolite~~** | **~~Endpoint (mg/kg dry soil)~~** | **~~PEC~~~~soil~~ ~~(mg/kg dry soil)~~** | **~~TER~~~~lt~~ ~~(criterion TER ≥ 10)~~** |
| ~~Azoxystrobin~~ | **~~LC~~~~50 CORR~~ ~~= 142~~** | ~~0.091~~ | **~~1560~~** |
| ~~R234886~~ | **~~LC~~~~50~~ ~~> 1000~~** | ~~0.022~~ | **~~> 45455~~** |
| ~~R402173~~ | **~~LC~~~~50~~ ~~> 1000~~** | ~~0.011~~ | **~~> 90909~~** |
| ~~R401553~~ | **~~LC~~~~50~~ ~~> 1000~~** | ~~0.007~~ | **~~> 142857~~** |
| **Chronic effects on earthworms** | | | |
| **Active substance/metabolite** | **Endpoint (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| azoxystrobin | NOEC = 20  **NOEC CORR = 10** | 0.091 \* | 220  **110** |
| ~~Metabolite, R234886~~ | **~~NOEC = 16~~** | ~~0.022~~ | **~~727~~** |
| **Chronic effects on other soil meso- and macrofauna** | | | |
| **Active substance/metabolite** | **Endpoint (mg a.s./kg dry soil)** | **PECsoil (mg/kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| **Collembola (*Folsomia candida*)** | | | |
| azoxystrobin | **NOEC = 50**  NOEC CORR = 25 | 0.091 \* | **549**  275 |
| **Soil mites (*Hypoaspis aculeifer*)** | | | |
| ~~azoxystrobin~~ | **~~NOEC = 1000~~**  ~~NOEC~~~~CORR~~ ~~= 500~~ | ~~0.091~~ ~~\*~~ | ~~10989~~  ~~5495~~ |

Values in **bold** are relevant for the conclusion of the risk assessment.

\* PECsoil, accu. For details please refer to section 8, chapter 8.7.

**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 use of BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | 2 x 2.0 L BAS 736 00 F/ha in cereals | | |
| **Chronic effects on earthworms** | | | |
| **Product** | **Endpoint (mg a.s./kg dry soil)** | **PECsoil (mg a.s./kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| (total) a.s. in BAS 736 00 F | NOEC= 33 1) EC10 = 34.3 1)  NOECCORR = 16.5 1) **EC10 CORR = 17.2 1)** | 0.169 \* 2) | 195  203  98  **102** |
| **Chronic effects on other soil meso- and macrofauna** | | | |
| **Product** | **Endpoint (mg a.s./kg dry soil)** | **PECsoil (mg a.s./kg dry soil)** | **TERlt (criterion TER ≥ 5)** |
| **Collembola (*Folsomia candida*)** | | | |
| (total) a.s. in BAS 736 00 F | NOEC= 17 1) **EC10 = 26.9 1)**  NOECCORR = 8.5 1) EC10 CORR = 13.5 1) | 0.169 \* 2) | 101  **159**  49  80 |
| **Soil mites (*Hypoaspis aculeifer*)** | | | |
| (total) a.s. in BAS 736 00 F | NOEC= 22.1 1) **EC10 = 50.9 1)**  NOECCORR = 11.1 1) EC10 CORR = 25.4 1) | 0.169 \* 2) | 131  **301**  66  150 |

Values in **bold** are relevant for the conclusion of the risk assessment.

\* PECsoil, accu. For details please refer to section 8, chapter 8.7.

1) Endpoint based on the content of the active substances (nominal) and taking into account a density of BAS 736 00 F of 1.078 g/cm3.

2) Based on the sum of the worst-case active substance PECsoil values.

#### Higher-tier risk assessment

Not relevant.

### Overall conclusions

**All TER values for BAS 736 00 F, the active substances fluxapyroxad, azoxystrobin and their relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the relevant trigger value. This indicates that BAS 736 00 F poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use pattern.**

|  |
| --- |
| **Review Comments:**  The long-term risks of BAS 736 00 F to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PECsoil. The relevant predicted environmental concentration in soil (PECsoil) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).  Safe use of BAS 736 00 F was confirmed based on TERLT calculations for formulation (endpoints based on the nominal content of the active substances), azoxystrobin and fluxapyroxad metabolites (M700F001 and M700F002). |

## Effects on soil microbial activity (KCP 10.5)

### Toxicity data

Studies on the effects on soil microorganisms have been carried out with the active substances fluxapyroxad, azoxystrobin and their relevant metabolites. Full details of these studies are provided in the respective EU documents.

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

All studies are listed in Table 9.9‑1, Table 9.9‑2 and Table 9.9‑3.

**Table 9.9‑1: Endpoints and effect values of fluxapyroxad and relevant metabolites relevant for the risk assessment for soil microorganisms**

| **Endpoint** | **Substance** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| N-mineralization | fluxapyroxad | 28 d, aerobic loamy sand soil | Nitrate formation rate 2.01 mg/kg dry soil -4.2% | EFSA Journal 2012; 10(1):2522 2008/1065108 |
| Metabolite, Reg. No. 5 069 089 M700F001 | 28 d, aerobic loamy sand soil | Nitrate formation rate 0.37 mg/kg dry soil +0.2% | EFSA Journal 2012; 10(1):2522 2008/1065121 |
| Metabolite, Reg. No. 5 435 595 M700F002 | 28 d, aerobic loamy sand soil | Nitrate formation rate 1.0 mg/kg dry soil -5.1% | EFSA Journal 2012; 10(1):2522 2009/1004145 |
| C-mineralization 1) | fluxapyroxad | 28 d, aerobic loamy sand soil | CO2 formation 2.01 mg/kg dry soil -4.2% | EFSA Journal 2012; 10(1):2522 2008/1065107 |
| Metabolite, Reg. No. 5 069 089 M700F001 | 28 d, aerobic loamy sand soil | CO2 formation 0.37 mg/kg dry soil -1.9% | EFSA Journal 2012; 10(1):2522 2008/1065120 |
| Metabolite, Reg. No. 5 435 595 M700F002 | 28 d, aerobic loamy sand soil | CO2 formation 1.0 mg/kg dry soil -1.6% | EFSA Journal 2012; 10(1):2522 2009/1004144 |

+ = stimulation, - = inhibition

1) Carbon transformation studies are listed for reference only but are not used in the risk assessment according to Commission Regulation (EU) No 283/2013.

**Table 9.9‑2: Endpoints and effect values of azoxystrobin and relevant metabolites relevant for the risk assessment for soil microorganisms**

| **Endpoint** | **Substance** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| N-mineralization | azoxystrobin  (tested as formulation 250 SC) | 28 d, aerobic loamy sand soil | Nitrate formation rate 3.3 mg/kg dry soil < 25% | ~~EC review report (1998), 7581/VI/97-Final,~~  EFSA Journal 2010; 8(4):1542,  Tarry, A.R., Prevett, A., Mason, G., 1994, ICI5504/0960 |
| Metabolite R234886 | 28 d, aerobic silty sand soil | Nitrate formation rate 10 mg/kg dry soil -3% | EFSA Journal 2010; 8(4):1542,  Lemnitzer, B., 2002, R234886/0002 |
| Metabolite R401553 | 28 d, aerobic loamy sand soil | Nitrate formation rate 2.643 mg/kg dry soil -0.1% | EFSA Journal 2010; 8(4):1542,  Schulz, L., 2008, SYN501657/0007 |
| Metabolite R402173 | 28 d, aerobic loamy sand soil | Nitrate formation rate 4.131 mg/kg dry soil +7.6% | EFSA Journal 2010; 8(4):1542,  Schulz, L., 2008a, SYN501114/0002 |
| C-mineralization 1) | Metabolite R234886 | 28 d, aerobic silty sand soil | CO2 formation 10 mg/kg dry soil -2% | EFSA Journal 2010; 8(4):1542,  Lemnitzer, B., 2002, R234886/0002 |
| Metabolite R401553 | 28 d, aerobic loamy sand soil | CO2 formation 2.643 mg/kg dry soil -4.4% | EFSA Journal 2010; 8(4):1542,  Schulz, L., 2008, SYN501657/0007 |
| Metabolite R402173 | 28 d, aerobic loamy sand soil | CO2 formation 4.131 mg/kg dry soil -0.4% | EFSA Journal 2010; 8(4):1542,  Schulz, L., 2008a, SYN501114/0002 |

+ = stimulation, - = inhibition

1) Carbon transformation studies are listed for reference only but are not used in the risk assessment according to Commission Regulation (EU) No 283/2013.

**Table 9.9‑3: Endpoints and effect values of BAS 736 00 F relevant for the risk assessment for soil microorganisms**

| **Endpoint** | **Product** | **Exposure System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| N-mineralization | BAS 736 00 F | 28 d, aerobic loamy sand | Nitrate formation rate at 35.00 mg/kg dry soil (equivalent to 1.6 mg fluxapyroxad and 2.4 mg azoxystrobin/kg dry soil) 1) +0.4% | not EU evaluated 2019/1061099 |

+ = stimulation, - = inhibition

1) Calculated, based on the nominal content of the a.s. and taking into account a density of BAS 736 00 F of 1.078 g/cm3.

#### Justification for new endpoints

Effects on soil microbial activity of BAS 736 00 F were not evaluated as part of the EU review of fluxapyroxad and azoxystrobin. Therefore, all relevant data and assessments are provided here and are considered adequate.

### Risk assessment

The evaluation of the risk for 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).

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).

The potential risk of BAS 736 00 F, fluxapyroxad, azoxystrobin and relevant metabolites to soil micro-organisms was assessed by comparing the maximum PECsoil values with the maximum concentration with effects ≤ 25 % (see Table 9.9‑4, Table 9.9‑5 and Table 9.9‑6).

**Table 9.9‑4: Assessment of the risk for effects on soil micro-organisms due to the use of fluxapyroxad as contained in BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | cereals | | |
| **Active substance** | fluxapyroxad | | |
| **Application rate (g a.s./ha)** | 2 x 100 | | |
| **N-mineralization** | | | |
| **Active substance/metabolite** | **Max. conc. with effects ≤ 25 % (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **Risk acceptable?** |
| fluxapyroxad | 2.01 (at 28 d) | 0.078 \* | yes |
| Metabolite, Reg. No. 5 069 089 M700F001 | 0.37 (at 28 d) | 0.004 \* | yes |
| Metabolite, Reg. No. 5 435 595 M700F002 | 1.0 (at 28 d) | 0.023 \* | yes |

\* PECsoil, accu. For details please refer to section 8, chapter 8.7.

**Table 9.9‑5: Assessment of the risk for effects on soil micro-organisms due to the use of azoxystrobin as contained in BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | cereals | | |
| **Active substance** | azoxystrobin | | |
| **Application rate (g a.s./ha)** | 2 x 150 | | |
| **N-mineralization** | | | |
| **Active substance/metabolite** | **Max. conc. with effects ≤ 25 % (mg/kg dry soil)** | **PECsoil (mg/kg dry soil)** | **Risk acceptable?** |
| azoxystrobin  (tested as formulation 250 SC) | 3.3 (at 28 d) | 0.091 \* | yes |
| Metabolite R234886 | 10 (at 28 d) | 0.022 | yes |
| Metabolite R402173 | 4.131 (at 28 d) | 0.011 | yes |
| Metabolite R401553 | 2.643 (at 28 d) | 0.007 | yes |

\* PECsoil, accu

**Table 9.9‑6: Assessment of the risk for effects on soil micro-organisms due to the use of BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intended use** | cereals | | |
| **Product** | BAS 736 00 F | | |
| **Application rate (L/ha)** | 2 x 2.0 | | |
| **N-mineralization** | | | |
| **Product** | **Max. conc. with effects ≤ 25 % (mg a.s./kg dry soil)** | **PECsoil (mg a.s./kg dry soil)** | **Risk acceptable?** |
| (total) a.s. in BAS 736 00 F | 4.0 (at 28 d) 1) | 0.169 \* 2) | yes |

\* PECsoil, accu for fluxapyroxad and azoxystrobin

1) Endpoint based on the content of the active substances (nominal) and taking into account the density of BAS 736 00 F of 1.078 g/cm³.

2) Based on the sum of the worst-case active substance PECsoil values.

### Overall conclusions

**For the formulation BAS 736 00 F, the active substances fluxapyroxad and azoxystrobin as well as for the relevant metabolites, the maximum concentration with effects < 25% (SANCO/10329/2002 trigger) are all above the maximum PECsoil values. Therefore, it is concluded that the use of BAS 736 00 F will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.**

|  |
| --- |
| **Review Comments:**  The use of BAS 736 00 F at the proposed rates poses no unacceptable risk to soil micro-organisms. |

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

### Toxicity data

Vegetative vigor and seedling emergence studies on the effects of the formulation BAS 736 00 F on non-target terrestrial plants were carried out which were not evaluated as part of the EU assessment of fluxapyroxad and azoxystrobin (see Table 9.10‑1).

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

**Table 9.10‑1: Endpoints and effect values of BAS 736 00 F relevant for the risk assessment for non-target terrestrial plants**

| **Species** | **Product** | **Exposure**  **System** | **Results** | **Reference** |
| --- | --- | --- | --- | --- |
| **Greenhouse** | | | | |
| *Daucus carota*d (carrot)  *Lactuca sativa*d (lettuce)  *Brassica oleracea*d(cabbage)  *Brassica napus*d (oilseed rape)  *Solanum lycopersicum* d (tomato)  *Glycine max* d (soybean)  *Allium cepa* m (onion)  *Lolium multiflorum* m (ryegrass)  *Triticum aestivum* m (wheat)  *Zea mays* m (corn) | BAS 736 00 F | 21 d 1) Seedling emergence | ER50 emergence > 2.0 L/ha  ER50 plant height > 2.0 L/ha  ER50 plant weight > 2.0 L/ha | not EU evaluated 2019/1061110 |
| *Daucus carota*d (carrot)  *Lactuca sativa*d (lettuce)  *Brassica oleracea*d(cabbage)  *Brassica napus*d (oilseed rape)  *Lycopersicon esculentum* d (tomato)  *Glycine max* d (soybean)  *Allium cepa* m (onion)  *Lolium multiflorum* m (ryegrass)  *Triticum aestivum* m (wheat)  *Zea mays* m (corn) | BAS 736 00 F | 21 d Vegetative vigor | ER50 plant height > 2.0 L/ha  ER50 plant weight > 2.0 L/ha | not EU evaluated  2019/1061112 |

m: monocotyledonous; d: dicotyledonous;

1) 28 days for carrot and onion.

#### Justification for new endpoints

Effects on non-target plants of BAS 736 00 F were not evaluated as part of the initial Annex I inclusion or the Annex I renewal process of fluxapyroxad and azoxystrobin. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

### Risk assessment

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

Not relevant.

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

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field areas, as non-target plants are non-cereals plants located outside the treated area.

The application of BAS 736 00 F is envisioned in cereals. The following risk assessment is based on the worst-case field application rate of 2.0 L BAS 736 00 F/ha (see Section 9 Chapter 9.1 for details).

The amount of spray drift reaching off-cereals habitats is calculated using the 90th percentile estimates in Appendix IV of ESCORT 2. Only a single application was considered, because factors like plant growth will reduce residues per unit area between multiple applications. The predicted rate reaching the off-cereals environment (PERoff-field) is calculated as:

For a single application to field crops (i.e.), 2.77% of the application rate was assumed to reach areas at 1 m from the edge of the field (worst-case scenario). The highest single application rate of BAS 736 00 F is 2.0 L product/ha. The maximum off-field predicted environmental rate (PERoff-field) is thus calculated to be 0.055 L product/ha.

The potential risk of BAS 736 00 F to non-target plants was assessed by comparing the calculated PER value to the ER50 values in order to generate the toxicity exposure ratio (TER) as follows.

The results of the risk assessment are presented in Table 9.10‑2.

**Table 9.10‑2: Assessment of the risk for non-target plants due to the use of BAS 736 00 F according to the proposed use pattern**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Intended use** | | cereals | | | |
| **Product** | | BAS 736 00 F | | | |
| **Application rate (L/ha)** | | 2 x 2.0 | | | |
| **MAF** | | n/a | | | |
| **Test species** | **ER50**  **(L/ha) 1)** | | **Drift rate**  **(%)** | **PERoff‑field**  **(L/ha)** | **TER**  **criterion: TER ≥ 5** |
| *Daucus carota*d (carrot)  *Lactuca sativa*d (lettuce)  *Brassica oleracea*d(cabbage)  *Brassica napus*d (oilseed rape)  *Lycopersicon esculentum* d (tomato)  *Solanum lycopersicum* d (tomato)  *Glycine max* d (soybean)  *Allium cepa* m (onion)  *Lolium multiflorum* m (ryegrass)  *Triticum aestivum* m (wheat)  *Zea mays* m (corn) | > 2.0 | | 2.77 | 0.055 | > 36.4 |

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio.

1) Worst caseendpoint derived from vegetative vigor and seedling emergence.

#### Higher-tier risk assessment

Not relevant.

#### Risk mitigation measures

No risk mitigation needed.

### Overall conclusions

**Based on the risk assessment it can be concluded that BAS 736 00 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 736 00 F applications are not required for the protection of terrestrial non-target plants.**

|  |
| --- |
| **Review Comments:**  The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002).  Based on the risk assessment it can be concluded that the proposed use of BAS 736 00 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 736 00 F applications are not required. |

## 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 736 00 F or of the active substances.

## Classification and Labelling

According to (EC) No 1272/2008 (CLP) plant protection products must be classified for their environmental hazard (acute and chronic). Classification is based on acute and chronic product data if adequate data is available. When sufficient product data is not available, the summation method is carried out instead.

For the product BAS 736 00 F acute data (LC/EC50) are available for all trophic levels. Regarding chronic toxicity, adequate data are only available for algae, thus chronic classification will be based on the summation method using data on the active substances. According to Annex VI of (EC) No 1272/2008 (CLP), the relevant chronic classification of active substance fluxapyroxad is H410 (M=1) and the relevant chronic classification of azoxystrobin is H410 (M=10). Table 9.13‑1 shows the relevant data for classification purposes.

**Table 9.13‑1: Ecotoxicology/Environment data relevant for classification of BAS 736 00 F**

| **Substance tested** | **Study Type**  **(duration)** | | **Findings** | | **Triggered classification and labelling** | | **Reference** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Acute (short-term) aquatic hazard** | | | | | | | |
| BAS 736 00 F | *Oncorhynchus mykiss* (96 h) | | LC50 = 2.83 mg/L | | No aquatic acute hazard cat. | | BASF DocID 2019/2039621 |
| BAS 736 00 F | *Daphnia magna* (48 h) | | EC50 = 1.57 mg/L | | No aquatic acute hazard cat. | | BASF DocID 2019/2039622 |
| BAS 736 00 F | *Pseudokirchneriella subcapitata*  (72 h) | | ErC50 = 4.19 mg/L | | No aquatic acute hazard cat. | | BASF DocID 2019/2039623 |
| ErC10 = 1.087 mg/L | | No aquatic chronic hazard cat. | |
| **Chronic (long-term) aquatic hazard** | | | | | | | |
| Fluxapyroxad (BAS 700 F) 1) | | *--* | | -- | Aquatic chronic hazard cat. 1 (H410); M=1 | Legal classification according to Annex VI of (EC) No 1272/2008 (CLP) | |
| Biodegradation | | not readily biodegradable | -- | BASF DocID 2008/1028082 | |
| Azoxystrobin 2) | | -- | | -- | Aquatic chronic hazard cat. 1 (H410); M=10 | Legal classification according to Annex VI of (EC) No 1272/2008 (CLP) | |
| Biodegradation | | No information available 3) | -- | EFSA Journal 2010; 8(4):1542 | |

1) Nominal contents within the formulated product: **50 g/L (4.64% w/w).**

2) Nominal contents within the formulated product: **75 g/L (6.96% w/w).**

3) According to the EFSA Conclusion of azoxystrobin (EFSA Journal 2010; 8(4):1542) no data on biodegradability is available. In a conservative approach, the active substance is assumed to be not readily biodegradable.

Based on the lowest acute aquatic toxicity endpoint obtained with BAS 736 00 F no aquatic acute hazard category is given according to (EC) No 1272/2008 (CLP).

Regarding chronic classification, fluxapyroxad (a.s. content of 4.64% w/w within the product), classified as chronic hazard cat. 1 (M=1) and azoxystrobin (a.s. content of 6.96% w/w within the product), classified as chronic hazard cat. 1 (M=10) are considered for the summation method in the 1stequation according to CLP (*M x hazard cat. 1*)*.* The 1st equation yields a value which is above the trigger of 25%. Hence, BAS 736 00 F is classified as aquatic chronic hazard category 1 (H410). Chronic classification of BAS 736 00 F using the summation method is summarized in Table 9.13‑2.

Table 9.13‑2: Chronic classification of BAS 736 00 F using the summation method according to (EC) No 1272/2008

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Chronic classification of BAS 736 00 F** | | | | | | | |
| Summation step | Name | Chronic category | M | Content in BAS 762 02 F [%] | Result (Content x M) | ∑ | Trigger |
| 1st equation 1) | Fluxapyroxad | 1 | 1 | 4.64 | 6.64 | 76.24 | > 25% |
| Azoxystrobin | 1 | 10 | 6.96 | 69.6 |

M = M-Factor

1) SUM *(M x Chronic 1)*

**Conclusion**

Based on the data obtained with the product and the lowest chronic aquatic toxicity endpoints of the classified compounds within the formulated product the following classification and labelling is proposed for BAS 736 00 F: **no** **aquatic acute hazard category and aquatic chronic hazard category 1 (H410)** according to GHS following Regulation (EC) No 1272/2008.

|  |
| --- |
| **Review Comments:**  The proposal of the classification of BAS 736 00 F is correct. |

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~~ | ~~Zok, S.~~ | ~~2009~~ | ~~BAS 700 F - Acute toxicity in the Zebra finch (Taeniopygia guttata) after single oral administration (LD50)~~  ~~2009/1050338~~  ~~BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~Yes~~ | ~~BASF~~ |
| KCP 10.1.1.1/2 | Hubbard, P. | 2020 | BAS 736 00 F: An acute oral toxicity study with the northern bobwhite using a sequential testing procedure  2020/2095640  Eurofins EAG Agroscience LLC, Easton MD, United States of America  yes  Unpublished | Yes | BASF |
| KCP 10.2.1/1 | Mingo, V. | 2020 | BAS 736 00 F: Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test – Static)  2019/2039621  Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | ~~No~~  Yes | BASF |
| KCP 10.2.1/2 | Lang, C. | 2020 | BAS 736 00 F: Toxicity to the water flea Daphnia magna Straus under laboratory conditions (Acute Immobilisation test - static)  2019/2039622  Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.2.1/3 | Obert-Rauser, P. | 2020 | BAS 736 00 F: Toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindák under Laboratory Conditions  2019/2039623  Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.1.1.1/1 | Wendling, K. | 2019 | BAS 736 00 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions  2019/1061095  Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| ~~KCP 10.3.1.1.1/2~~ | ~~Verge, E.~~ | ~~2014~~ | ~~BAS 700 04 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions~~  ~~2014/1135445~~  ~~Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | ~~BASF~~ |
| KCP 10.3.1.1.2/1 | Wendling, K. | 2019 | BAS 736 00 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions  2019/1061095  Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| ~~KCP 10.3.1.1.2/2~~ | ~~Verge, E.~~ | ~~2014~~ | ~~BAS 700 04 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions~~  ~~2014/1135445~~  ~~Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | ~~BASF~~ |
| ~~KCP 10.3.1.2/1~~ | ~~Dressler, K.~~ | ~~2021~~ | ~~Chronic toxicity of BAS 700 F tested as BAS 700 04 F to the honey bee Apis mellifera L. under laboratory conditions~~  ~~2020/2083866~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | ~~BASF~~ |
| KCP 10.3.1.2/2 | Haeuser, R. | 2021 | BAS 736 00 F - Honey Bee (Apis mellifera L.) Chronic Oral Toxicity Test - 10 Day Feeding Test in the Laboratory  2020/2080999  Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| ~~KCP 10.3.1.3/1~~ | ~~Kleebaum, K.~~ | ~~2018~~ | ~~Repeated exposure of honey bee (Apis mellifera) larvae to BAS 700 F (Fluxapyroxad) under laboratory conditions (in vitro)~~  ~~2017/1036679~~  ~~BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | ~~BASF~~ |
| KCP 10.3.1.3/2 | Haeuser, R. | 2021 | Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 736 00 F under laboratory conditions  2020/2081000  Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.2.1/1 | Roehlig, U. | 2019 | Effects of BAS 736 00 F on the predatory mite Typhlodromus pyri SCHEUTEN in a laboratory test  2019/1061102  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  no  Unpublished | No | BASF |
| KCP 10.3.2.1/2 | Roehlig, U. | 2019 | Effects of BAS 736 00 F on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in a laboratory test  2019/1061100  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.2.2/1 | Roehlig, U. | 2019 | Effects of BAS 736 00 F on the predatory mite Typhlodromus pyri Scheuten in an extended laboratory test  2019/1061104  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.2.2/2 | Roehlig, U. | 2019 | Effects of BAS 736 00 F on the parasitic wasp Aphidius rhopalosiphi (Destefani-Perez) in an Extended laboratory test  2019/1061101  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.2.2/3 | Roehlig, U. | 2020 | Effects of BAS 736 00 F on the green lacewing Chrysoperla carnea STEPH. in an (extended laboratory test)  2019/2054735  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| ~~KCP 10.4.1.1/1~~ | ~~Witte, B.~~ | ~~2014~~ | ~~Effects of BAS 700 F (Fluxapyroxad) on reproduction and growth of earthworms Eisenia fetida in artificial soil~~  ~~2014/1135449~~  ~~Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | ~~BASF~~ |
| KCP 10.4.1.1/2 | Friedrich, S. | 2019 | Effects of BAS 736 00 F on the reproduction of the earthworm Eisenia andrei in artificial soil  2019/1061096  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| ~~KCP 10.4.2.1/1~~ | ~~Friedrich, S.~~ | ~~2014~~ | ~~Effects of BAS 700 F (Fluxapyroxad) on the reproduction of the collembolan Folsomia candida~~  ~~2014/1135433~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | ~~BASF~~ |
| ~~KCP 10.4.2.1/2~~ | ~~Schulz, L.~~ | ~~2016~~ | ~~Effects of BAS 700 F (Fluxapyroxad) on the reproduction of the predatory mite Hypoaspis aculeifer~~  ~~2016/1122885~~  ~~BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.~~  ~~yes~~  ~~Unpublished~~ | ~~No~~ | ~~BASF~~ |
| KCP 10.4.2.1/3 | Friedrich, S. | 2019 | Effects of BAS 736 00 F on the reproduction of the collembolan Folsomia candida  2019/1061097  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.4.2.1/4 | Schulz, L. | 2021 | Effects of BAS 736 00 F on the reproduction of the predatory mite Hypoaspis aculeifer  2019/1061098  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.5/1 | Persdorf, M. | 2019 | Effects of BAS 736 00 F on the activity of soil microflora (Nitrogen transformation test)  2019/1061099  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.6.2/1 | Maleck, A. | 2019 | Effect of BAS 736 00 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions  2019/1061112  Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.6.2/2 | Maleck, A. | 2019 | Effect of BAS 736 00 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions  2019/1061110  Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep.  yes  Unpublished | No | BASF |

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

BAS 736 00 F is a new product, no product data have been evaluated previously.

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 10.1.1.1/1 | Zok, S. | 2009 | BAS 700 F - Acute toxicity in the Zebra finch (Taeniopygia guttata) after single oral administration (LD50)  2009/1050338  BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.  yes  Unpublished | Yes | BASF |
| KCP 10.3.1.1.1/2 | Verge, E. | 2014 | BAS 700 04 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions  2014/1135445  Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.1.1.2/2 | Verge, E. | 2014 | BAS 700 04 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions  2014/1135445  Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.1.2/1 | Dressler, K. | 2021 | Chronic toxicity of BAS 700 F tested as BAS 700 04 F to the honey bee Apis mellifera L. under laboratory conditions  2020/2083866  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.3.1.3/1 | Kleebaum, K. | 2018 | Repeated exposure of honey bee (Apis mellifera) larvae to BAS 700 F (Fluxapyroxad) under laboratory conditions (in vitro)  2017/1036679  BioChem agrar GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.4.1.1/1 | Witte, B. | 2014 | Effects of BAS 700 F (Fluxapyroxad) on reproduction and growth of earthworms Eisenia fetida in artificial soil  2014/1135449  Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.4.2.1/1 | Friedrich, S. | 2014 | Effects of BAS 700 F (Fluxapyroxad) on the reproduction of the collembolan Folsomia candida  2014/1135433  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |
| KCP 10.4.2.1/2 | Schulz, L. | 2016 | Effects of BAS 700 F (Fluxapyroxad) on the reproduction of the predatory mite Hypoaspis aculeifer  2016/1122885  BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.  yes  Unpublished | No | BASF |

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: | Study not evaluated. |

Overall, three acute toxicity studies in birds are available for the active substance fluxapyroxad. Two standard acute toxicity studies with bobwhite quail and mallard duckwhich have already been evaluated during the Annex I inclusion process. In addition, a new acute study on zebra finch (*Taeniopygia guttata*) was conducted. The study has already been used in previous EU end-use product registrations but has not been evaluated previously on EU level and is provided in support of the bird risk assessment.

|  |  |
| --- | --- |
| *Reference:* | *CP 10.1.1.1/1* |
| *Report* | *BAS 700 F - Acute toxicity in the Zebra finch (Taeniopygia guttata) after single oral administration (LD50),*  ***Zok S., 2009***  *report No 15W0683/055095*  *BASF DocID 2009/1050338*  *Authority registration No* |
| *Guideline(s):* | *EPA 71-1, EPA 850.2100, EPA 540/9-82-024, EPA 540/9-85-007, EPA 712-C-96-139* |
| *Deviations:* | *No* |
| *GLP:* | *yes*  *(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)* | | |
| *Acceptability:* | *Yes* | |
| *Duplication  (if vertebrate study)* | *No* | |

**Executive Summary**

An acute oral avian toxicity test with BAS 700 F was conducted. The objective of the study was to test the item on its acute toxicity in the zebra finch and to determine the LD50 and the no observed effect level (NOEL). The test item was administered via a single-dose of 500, 1000 or 2000 mg BAS 700 F/kg b.w. to 1 – 2 month old zebra finches. Ten birds (5 males and 5 females) were used in each group. The doses were suspended in 0.5% aqueous carboxyl methyl cellulose as a carrier which was applied once by gavage in the crop. Feed was removed for 19 - 21 hours prior to dosing.

All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days post dosing. Afterwards a gross post-mortem examination was conducted. All groups received food and water ad libitum throughout the test. The test was terminated after 14 days.

In males, no mortality and no toxic signs were observed in the control and any of the test item treatments up to the highest tested concentration of 2000 mg BAS 700 mg/kg b.w..

No substance-related impairment of feed uptake in comparison to the control was observed in any of the dose groups. No statistically significant substance-related reduction of the body weights in the male and female birds was observed on day 7 and at day 14 (sacrifice) in any of the dose groups compared to the control and the body weight development was not impaired compared to the control group. No substance-related abnormalities were detected in surviving birds after sacrifice. In one female bird of the control group a clay-like coloration of the liver was observed.

**In an acute toxicity test with the zebra finch, the LD50 of BAS 700 F was > 2000 mg/kg b.w.. The NOEL was ≥ 2000 mg/kg b.w..**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 700 F; batch no. COD-001049 content of a.s.: BAS 700 F, (Reg. No. 5094351)

**B. STUDY DESIGN**

Test species: Zebra finch (*Taeniopygia guttata*); indistinguishable from wild birds; age: approx. 1 - 2 month; source: Kölle-Zoo, Ludwigshafen, Germany

Test design: Birds were administered different doses of the test item BAS 700 F with the carrier of 0.5% aqueous carboxy methyl cellulose suspension in bidistilled water, in a total amount of 10 g per kg body weight by gavage into the crop; 5 males and 5 females per dose group were used; observation period of 14 days; assessment of mortality and signs of clinical toxicity was carried out three times on day of dosing, daily thereafter; assessment of body weight was carried out on day 7 and 14. Gross-mortem examinations of all birds at termination of the test were conducted.

Endpoints: LD50, mortality, clinical signs, feed consumption, body weight (b.w.), gross pathological examinations were conducted on all birds sacrificed at the termination of the test.

Test concentrations: Control, 500, 1000 and 2000 mg BAS 700 mg a.s./kg b.w.

Test conditions: Birds fasted for about 19 - 21 h before administration of the test item; temperature 20.0 °C to 24.4 °C, deviation for 2 days, 21 h and 45 min from the temperature range of 20.8 °C – 23.4 °C; relative humidity: 45% - 75%, deviation of 1 day, 15 h and 30 minutes above the limit of 45% - 70%; deviation of 22 h and 15 minutes below the limit of 45% - 70%; photoperiod: 8 hours light : 16 hours dark.

Analytics: The test substance concentrations were analysed using HPLC.

Statistics: Descriptive statistics, Dunnett-test for body weight data (α = 0.05).

**C. Description of the analytical procedures**

Concentrations of BAS 700 F in 0.5% aqueous carboxy methyl cellulose were determined using the method described within the study report. The samples were diluted with acetonitrile/highly deionized water 1/1 (v/v) into measuring flasks and filled up to a total volume of 100mL (sample 1) and 200mL (sample 2, 3). The sample 3 was further diluted with acetonitrile/highly deionized water 1/1 (v/v) at a ratio of 1:1. Aliquots of the samples were used for HPLC- analysis. The determination was performed by HPLC-UV detection. The limit of quantification (LOQ) was 164.5 mg/L and the limit of detection (LOD) was not given. Details on measured fortification samples and obtained procedural recoveries for BAS 700 F are given in the table below.

**Table A 1: Procedural recoveries for BAS 700 F**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Matrix** | **Fortification Level [g/50g]** | **BAS 700 F** | | | |
| **n** | **Mean [%]** | **SD [±]** | **RSD [%]** |
| Carboxy methyl cellulose | 2.5 | 2 | 92.1 | 0.019 | 0.83 |
| Carboxy methyl cellulose | 5.0 | 2 | 95.2 | 0.030 | 0.64 |
| Carboxy methyl cellulose | 10.1 | 2 | 95.8 | 0.057 | 0.58 |

**II. RESULTS AND DISCUSSION**

***Analytical results:***

The values of the analytically determined concentrations of the preparations given to the birds by gavage were close to the nominal concentrations (92 – 96%). The biological results are therefore based on the nominal values.

***Biological results:***

In both groups males and females, no mortality and no toxic signs were observed in the control and any of the test item treatment groups up to the highest tested concentration of 2000 mg /kg b.w..

Diarrhea in the first time after dosing is a consequence of the fasting period and is usually observed in all dose groups as well as in the control group and is not considered to be toxic effect.

No substance-related impairment of feed uptake in comparison to the control was observed in any of the dose groups. No statistically significant substance-related reduction of the body weights was observed in any of the dose groups at day 7 or day 14 and the body weight development was not impaired in comparison to the control group.

No treatment-related macroscopic abnormalities were detected in the gross post-mortem examination. The relevant endpoints are summarized in Table A 2.

**Table A 2: Acute toxicity of BAS 700 F to the zebra finch (*Taeniopygia guttata*)**

| **Mortality** | **Dose [mg BAS 700 F /kg b.w.]** |
| --- | --- |
| Highest dose causing no treatment-related mortality | males: 2000  females: 1000 |
| LD50 (14 d) | > 2000 |
| NOEL | ≥ 2000 |

b.w. = body weight

Validity criteria:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Criteria** | **Trigger value** | **Study value and page no.** | **Criteria met? Y/N** | **Guideline** |
| Birds assignment to treatment and control pens | Random | Random  p. 16 | Y | OCSPP 850.2100 |
| Control birds died or became moribund | ≤ 10% | 0%  p. 25 | Y | OCSPP 850.2100 |
| Number of birds used for each dose level of the test substance and control | ≥ 10 | 10  p. 19 | Y | OCSPP 850.2100 |
| Test substance method of administration | Orally, by capsule or gavage | Gavage  p. 22 | Y | OCSPP 850.2100 |
| In a definitive test only, number of treatments tested | ≥ 5 + control | 3 + control  p. 19 | N | OCSPP 850.2100 |
| Non-incidental control mortality1 | 0% | 0%  p. 25 | Y | OECD 223 |
| Incidental control mortality1 | ≤ 10% | 0%  p. 25 | Y | OECD 223 |

1Incidental = self-inflicted, e.g., abrasions or broken legs. Non-incidental mortality is things like disease or mishandling of animals that indicate poor health of the test population or poor study conduct.

In Commission Regulation (EU) No. 283/2013, acute bird studies are required to be conducted using either US EPA OCSPP 850.2100 (2012) or OECD 223 (2010). This study was conducted under EPA 71-1 and EPA OPPTS 850.2100, which are precursors of OCSPP 850.2100. All validity criteria for OCSPP 850.2100 and OECD 223 (included for completeness) are fulfilled except one. While this study tested 3 treatments + control, OCSPP 850.2100 requires 5 treatments + control. However, no mortality occurred in this study and the maximum dose of 2000 mg/kg b.w. recommended by OCSPP 850.2100 was tested. Therefore, the results of this study would not have been altered by the inclusion of more dose levels below 2000 mg/kg b.w. and this study should be considered fully valid for use in risk assessment.

**III. CONCLUSION**

**In an acute toxicity test with the zebra finch, the LD50 of BAS 700 F was > 2000 mg/kg b.w.. The NOEL was ≥ 2000 mg/kg b.w..**

* + - * 1. Study 2

|  |  |
| --- | --- |
| 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 10.1.1.1/2* |
| *Report:* | *BAS 736 00 F: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure*  *Hubbard P.M., Temple D.L., 2020*  *Study no. 147B-38, 849610*  *BASF DocID:* *2020/2095640* |
| *Guideline(s):* | *OECD Guideline 223* |
| *Deviations:* | *No* |
| *GLP:* | *Yes*  *(certified by United States Environmental Protection Agency)* | |
| *Acceptability:* | *Yes* |
| *Duplication  (if vertebrate study):* | *No* |

**Executive Summary**

An acute oral avian toxicity test with the formulation BAS 736 00 F containing the active substances fluxapyroxad and azoxystrobin was conducted. The objective of the study was to provide evidence that the LD50 for the test substance is above the tested limit-dose.

The test substance was administered via a single oral dose of 0 (control) and 2000 mg formulation/kg b.w. to 49-week-old bobwhite quails. Five birds (mixed sex) were used in each test group. After fasting for approximately 17.6 hours, the test substance was administered once by gavage directly into the crop of each bird at the dosing volume of 1.86 mL (in reverse osmosis deionized water) per kg body weight. The animals of the control group received reverse osmosis deionized water only and all birds were observed for regurgitation on the Day 0 of the test. The birds were offered Easton game bird ration *ad libitum* with the exception of a fasting period prior to dosing and municipal water *ad libitum*. The test was terminated after 14 days. Mortalities, signs of toxicity and abnormal behavior were recorded at least twice daily during the observation period. Gross-pathological examinations were conducted on all birds.

No regurgitation was observed among the control birds or among any of the treatment birds. No mortality and no toxic signs were observed in the control and the test item treatment of 2000 mg BAS 736 00 F/kg b.w. and all birds were normal in appearance and behavior for the duration of the test.

When compared to the control group, there was a statistically significant treatment related loss of mean body weight in the 2000 mg BAS 736 00 F/kg dosage level from Day 0 to Day 3. There were no other statistically significant differences in mean body weight or mean body weight changes for the 2000 mg BAS 736 00 F/kg group when compared to the control group. There was a reduction in feed consumption from Day 0 to 1 and Day 1 to 2 for the 2000 mg BAS 736 00 F/kg dosage level relative to the control group. For all feeding intervals from Day 2 to Day 14 the feed consumption for the 2000 mg BAS 736 00 F/kg dosage group was comparable to the control group.

No test substance specific effects were identified during the gross post-mortem examination.

**In an acute toxicity test with the bobwhite quail (*Colinus virginianus*), the 14-day LD50 of BAS 736 00 F was > 2000 mg BAS 736 00 F/kg b.w.. The NOEL for mortality was 2000 mg BAS 736 00/kg b.w..**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no.: FD-190220-0002; content of a.s.: Fluxapyroxad (BAS 700 F, Reg. No. 5094351): 49.4 g/L (nominal: 50 g/L), Azoxystrobin (BAS 9164 F, Reg. No. 300254): 73.9 g/L (nominal: 75 g/L).

**B. STUDY DESIGN**

Test species: Bobwhite quail (*Colinus virginianus*), visually indistinguishable from wild birds; age: 49 weeks at dosing, hatch date: 16 July 2019; source: Trace Pheasantry, Douglassville, Pennsylvania, United States.

Test design: Five birds of mixed sex were randomly assigned each to the treatment group and the control group. Prior to the administration, the birds were fasted for approx. 17.6 hours. Thereafter birds were administered a single dose, once by gavage into the crop in a total amount of 1.86 mL/kg b.w. of 0 (reverse osmosis deionized water) or 2000 mg BAS 736 00 F/kg b.w (in reverse osmosis deionized water). The animals were observed for regurgitation on Day 0 of the test. An observation period of 14 days followed, during which mortalities, signs of toxicity and abnormal behavior were observed at least twice daily. Body weights were measured individually on the day of dosing (Day 0) and on Days 3, 7, and 14 of the test. Food consumption was determined by pen for approx. 24-hour intervals from Day 0 to 1, Day 1 to 2, and Day 2 to 3. Mean food consumption (g/animal/day) was then determined from Days 3 to 7 and from Days 7 to 14. Mean food consumption was calculated by measuring the change in weight of the food offered to the birds over a given period of time. All animals were sacrificed by CO2 asphyxia at the termination of the observation period. The post-mortem examination included, but was not limited to, a general examination of the exterior of the bird, and an examination of the thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastro-intestinal tract, and urogenital system.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations were conducted. Calculation of LD50 and NOEL was conducted.

Test concentrations: 0 (control) and 2000 mg formulation/kg b.w.

Test conditions: Birds fasted for approx. 17.6 hours before administration of the test substance; average temperature: 21.1°C, between 20.5°C (minimum) and 21.9°C (maximum); average relative humidity: 72%, between 58% (minimum) and 80% (maximum); photoperiod: 8 hours light : 16 hours dark, fluorescent lights that closely approximated the color spectrum of noonday sunlight. Light intensity: approx. 124 Lux.

Analytics: The analysis of the test item preparations has not been performed.

Statistics: Descriptive statistics; body weight change: Student’s t-test. No statistical calculation of the LD50 was performed since the test was run as a limit test with only one control and one dose group

**C. Description of the analytical procedures**

Not applicable, no analysis of the test-substance preparations was carried out.

**II. RESULTS AND DISCUSSION**

***Biological results:***

No regurgitation was observed among the control birds or among any of the treatment birds. No mortality and no toxic signs were observed in the control and the test item treatment of 2000 mg BAS 736 00 F/kg b.w. and all birds were normal in appearance and behavior for the duration of the test.

When compared to the control group, there was a statistically significant treatment related loss of mean body weight in the 2000 mg BAS 736 00 F/kg dosage level from Day 0 to Day 3. There were no other statistically significant differences in mean body weight or mean body weight changes for the 2000 mg BAS 736 00 F/kg group when compared to the control group. There was a reduction in feed consumption from Day 0 to 1 and Day 1 to 2 for the 2000 mg BAS 736 00 F/kg dosage level relative to the control group. For all feeding intervals from Day 2 to Day 14 the feed consumption for the 2000 mg BAS 736 00 F/kg dosage group was comparable to the control group.

No test substance specific effects were identified during the gross post-mortem examination.

**Table A 3: Acute toxicity of BAS 736 00 F to the bobwhite quail (*Colinus virginianus*)**

|  |  |
| --- | --- |
| **Mortality** | **Dose [mg BAS 736 00 F/kg b.w.]** |
| Highest dose causing no treatment-related mortality | 2000 |
| NOEL mortality | 2000 |
| LD50 (14 d) | > 2000 |

b.w. = body weight

**Validity criteria:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Criteria** | **Trigger value** | **Study value and page no.** | **Criteria met? Y/N** | **Guideline** |
| Non-incidental control mortality1 | 0% | 0%  p. 16 | Y | OECD 223 |
| Incidental control mortality1 | ≤ 10% | 0%  p. 16 | Y | OECD 223 |

1Incidental = self-inflicted, e.g., abrasions or broken legs. Non-incidental mortality is things like disease or mishandling of animals that indicate poor health of the test population or poor study conduct.

In Commission Regulation (EU) No. 283/2013, acute bird studies are required to be conducted using either US EPA OCSPP 850.2100 (2012) or OECD 223 (2016). This study was conducted under OECD 223 (2016) and met all validity criteria for OECD 223.

Therefore, this study should be considered fully valid and reliable for use in the risk assessment.

**III. CONCLUSION**

**In an acute toxicity test with the bobwhite quail, the 14-day LD50 of BAS 736 00 F was > 2000 mg BAS 736 00 F/kg b.w.. The NOEL for mortality was 2000 mg BAS 736 00 F/kg b.w.**

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

No studies conducted

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

No further studies conducted.

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

No studies conducted.

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

No studies 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.  The results refer to nominal concentrations. |

|  |  |
| --- | --- |
| Reference: | CP 10.2.1/1 |
| Report | BAS 736 00 F: Toxicity to the Rainbow Trout Oncorhynchus mykiss under Laboratory Conditions (Acute Toxicity Test – Static),  Mingo, V., 2020  report No 849663, S19-20423  2019/2039621  Authority registration No |
| Guideline(s): | OECD 203 (2019) |
| Deviations: | No |
| GLP: | yes  (certified by LUBW Landesanstalt fuer Umwelt Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes/ |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a 96-hour static acute toxicity laboratory study, juvenile rainbow trout were exposed to nominal concentrations of 0 (control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg BAS 736 00 H/L in groups of 7 animals per treatment in glass aquaria containing 15 L of water. Fish were observed for survival and symptoms of toxicity after 0 h, 4 – 6 h, and once or twice daily thereafter until test end (96 h).

The biological results are based on nominal test item concentrations. After 96 hours of exposure, no mortality was observed in the control and at concentrations of up to and including 2.0 mg BAS 736 00 F/L, whereas 100% mortality was observed at the two highest test item concentrations. Sublethal effects could not be observed at test end, but at earlier timepoints.

**In a 96-hour static acute toxicity study on rainbow trout, the LC50 (96 h) of BAS 736 00 F was 2.83 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 2.0 mg/L (nominal).**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F, batch no. FD-190220-0002, content of a.s.: azoxystrobin (BAS 9164 F, reg. no. 300254: 73.9 g/L (nominal: 75.0 g/L), fluxapyroxad (BAS 700 F, reg. no. 5094351): 49.4 g/L (nominal: 50.0 g/L); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Rainbow trout (*Oncorhynchus mykiss,* Walbaum); average body length: 47 ± 3 mm (test end); average body weight: 1.45 ± 0.23 g (test end); supplied by trout farm "Peter Störk", D-88348 Bad Saulgau, Germany.

Test design: Static system (96 hours); 5 test item concentrations plus a control with untreated test medium, 1 replicate (aquarium) per treatment; 7 fish per treatment and aquarium (loading 0.68 g fish/L); assessment of mortality and symptoms of toxicity after 0 h, 4 – 6 h, 1 day, 2 days (twice daily), 3 days (twice daily) and 4 days after start of exposure.

Endpoints: LC50, NOEC, based on mortality and sub-lethal effects.

Test concentrations: 0 (control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg BAS 736 00 F/L (nominal).

Test conditions: Test vessels: 18-L glass aquaria, test volume: 15 L; test medium: dechlorinated drinking water mixed with deionized water; temperature: 12.5 – 13.4 °C; pH 7.61 – 8.10; oxygen content: 70 – 97% of air saturation; conductivity: 414 µS/cm; hardness: 178 mg CaCO3/L; photoperiod: 16 h light : 8 h dark with 30 min transition; light intensity: 879 lux (at test start); no aeration, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS/MS-detection (analytical method no. L0361/03 and L0361/01).

Statistics: Descriptive statistics; 96-h LC50 value was determined by the geometric mean between the highest test item concentration causing no mortality and the lowest test item concentration causing 100 % mortality; Step-down Cochran-Armitage test (α = 0.05) for determination of NOEC.

**C.** **DESCRIPTION OF THE ANALYTICAL PROCEDURES**

Concentrations of fluxapyroxad (BAS 700 F; contained in BAS 736 00 F) and azoxystrobin (BAS 9164 F; contained in BAS 736 00 F) in test medium were determined according to the analytical methods L0361/01 and L0361/03, respectively. The validation of each analytical methods is described within separate study reports BASF DocID 2017/1065621 and 2019/1039564, respectively. Analysis of BAS 700 F and BAS 9164 F was performed by direct injection of test medium samples after dilution with acetonitrile/water/formic acid (400/600/2, v/v/v) and if necessary further dilution with diluted blank medium (test medium /(acetonitrile/water/formic acid (400/600/2, v/v/v)) (1:1, v/v)) before analysis. Final determination was accomplished by LC-MS/MS. The limit of quantification (LOQ) was 0.00229 mg/L for fluxapyroxad and 0.00343 mg/L for azoxystrobin. The limit of detection (LOD) was set to 0.000597 mg/L for fluxapyroxad and 0.000897 mg/L for azoxystrobin. In the current study, matrix effects on LC-MS/MS detection were not investigated. Matrix-matched standards were used for quantification. In the current study, the maximum storage period from sampling to analysis was 12 days for BAS 700 F and B AS 9164 F. Residues are regarded as stable if the samples are stored deep frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95). Therefore, the stability of BAS 700 F and BAS 9164 F in BAS 736 00 F was not verified. The average recoveries for the fortification levels of 0.05 and 10.4 mg/L were found to be 103% and 108% for fluxapyroxad and 102% – 104% for azoxystrobin.

Details on measured fortification samples and obtained procedural recoveries are provided in the tables below.

**Table A 4: Procedural recoveries for BAS 736 00 F (based on measurement of BAS 700 F)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level  [mg BAS 700 F/L]** | **n** | **Mean recovery [%]** | **RSD [%]** |
| Test medium | 0.05 | 5 | 103 | 4 |
| Test medium | 10.4 | 5 | 108 | 3 |

Abbreviation: RSD = relative standard deviation

**Table A 5: Procedural recoveries for BAS 736 00 F (based on measurement of BAS 9164 F)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level  [mg BAS 9164 F/L]** | **n** | **Mean recovery [%]** | **RSD [%]** |
| Test medium | 0.05 | 5 | 102 | 4 |
| Test medium | 10.4 | 5 | 104 | 3 |

Abbreviation: RSD = relative standard deviation

**II. RESULTS AND DISCUSSION**

Analytical measurements: Analytical verification of azoxystrobin and fluxapyroxad concentrations was conducted in each concentration at the beginning and at the end of the test. After 24 and 72 h analytical verification was conducted for 8.0 mg/L and 4.0 mg/L, respectively. The analyzed contents of azoxystrobin ranged from 96.7% to 101% of nominal concentrations at test initiation, was determined to be 93.1% of nominal for the concentration of 8.0 mg/L after 24 h, 93.4% of nominal for the concentration of 4.0 mg/L after 72 h and ranged from 90.9% to 96.4% of nominal at test termination. The analyzed contents of fluxapyroxad ranged from 97.8% to 100% of nominal concentrations at test initiation, was determined to be 92.9% of nominal for the concentration of 8.0 mg/L after 24 h, 93.4% of nominal for the concentration of 4.0 mg/L after 72 h and ranged from 88.5% to 100% of nominal at test termination. The following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the control and at concentrations of up to and including 2.0 mg BAS 736 00 F/L, whereas 100% mortality was observed at the two highest test item concentrations. Sublethal effects could not be observed at test end, but at earlier timepoints. The results are summarized in Table A 6.

**Table A 6: Acute toxicity (96 h) of BAS 736 00 F on rainbow trout (*O. mykiss*)**

| **Concentration [mg/L] (nominal)** | **Control** | **0.5** | **1.0** | **2.0** | **4.0** | **8.0** |
| --- | --- | --- | --- | --- | --- | --- |
| Mortality [%] | 0 | 0 | 0 | 0 | 100\* | 100\* |
| Symptoms1) | none | none | none | none | n.d. | n.d. |
|  | **Endpoints [mg BAS 736 00 F/L] (nominal)** | | | | | |
| LC50 (96 h) | 2.83 (95% confidence limits: n.c.) | | | | | |
| NOEC (96 h) | 2.0 | | | | | |

n.d. = not determined; all fish dead; n.c. = not calculated due to mathematical reasons.

\* Statistically significant difference compared to control (step-down Cochran-Armitage, α = 0.05)

1) Symptoms have only been observed in earlier time points. Fish either recovered (2.0 mg/L) or died (4 mg/L) until test end.

|  |  |
| --- | --- |
| **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 | > 60%  (70 – 97%) |

**III. CONCLUSION**

**In a 96-hour static acute toxicity study on rainbow trout, the LC50 (96 h) of BAS 736 00 F was 2.83 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 2.0 mg/L (nominal).**

* + - 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.  The results refer to nominal concentrations. |

|  |  |
| --- | --- |
| Reference: | CP 10.2.1/2 |
| Report | BAS 736 00 F: Toxicity to the water flea Daphnia magna Straus under laboratory conditions (Acute Immobilisation test - static),  Lang, C., 2020  report No 849662, S19-20422  2019/2039622  Authority registration No |
| Guideline(s): | OECD 202 (2004) |
| Deviations: | No |
| GLP: | yes  (certified by LUBW Landesanstalt fuer Umwelt Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to BAS 736 00 F at nominal concentrations of 0 (control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg BAS 736 00 H/L in 4 replicates per concentration, containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after the start of exposure.

The biological results are based on nominal concentrations. After 48 hours of exposure no immobility occurred at nominal concentrations up to and including 1.0 mg BAS 736 00 F/L, whereas 85, 100 and 100% of the daphnids were immobile at 2.0, 4.0 and 8.0 mg BAS 736 00 F/L, respectively, which was a statistically significant increase in immobility compared to the control. No other sublethal effects were observed in the control and all test item concentrations during the test.

**In a 48-hour static acute toxicity study on *Daphnia magna,* the EC50 (48 h) of BAS 736 00 F was 1.57 mg/L based on nominal concentrations. The NOEC was determined to be 1.0 mg/L (nominal).**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F, batch no. FD-190220-0002, content of a.s.: azoxystrobin (BAS 9164 F, reg. no. 300254: 73.9 g/L (nominal: 75.0 g/L), fluxapyroxad (BAS 700 F, reg. no. 5094351): 49.4 g/L (nominal: 50.0 g/L); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Water flea (*Daphnia magna* STRAUS), clone V, not first-brood progeny, neonates collected from in house culture less than 24 hours old at test initiation, originally obtained from Federal Environmental agency, Berlin, Germany.

Test design: Static system (48 hours), 5 test item concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC50 based on immobility of daphnids.

Test concentrations: 0 (control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg BAS 736 00 F/L (nominal).

Test conditions: 100 mL glass beakers, test volume: 50 mL, test medium: dilution water "M4" (Elendt medium); pH 7.71 – 8.10; oxygen content: 7.6 mg/L – 8.9 mg/L; temperature: 20.1 °C – 20.5 °C, photoperiod: 16 h light : 8 h dark; light intensity: ~630 lux; no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a LC-method with MS/MS-detection (methods no. L0361/03 and L0361/01).

Statistics: Descriptive statistics; Trimmed Spearmen-Karber Procedure for determination of the EC50 value; Step-down Cochran-Armitage Test Procedure for determination of the NOEC.

**C. DESCRIPTION OF THE ANALYTICAL PRODECDURES**

Concentrations of fluxapyroxad (BAS 700 F; contained in BAS 736 00 F) and azoxystrobin (BAS 9164 F; contained in BAS 736 00 F) in test medium were determined according to the analytical methods L0361/01 and L0361/03, respectively. The validation of each analytical methods is described within separate study reports BASF DocID 2017/1065621 and 2019/1039564, respectively. Analysis of BAS 700 F and BAS 9164 F was performed by direct injection of test medium samples after dilution with acetonitrile/water/formic acid (400/600/2, v/v/v) and if necessary further dilution with diluted blank medium (test medium /(acetonitrile/water/formic acid (400/600/2, v/v/v)) (1:1, v/v)) before analysis. Final determination was accomplished by LC-MS/MS. The limit of quantification (LOQ) was 0.00229 mg/L for fluxapyroxad and 0.00343 mg/L for azoxystrobin. The limit of detection (LOD) was set to 0.000597 mg/L for fluxapyroxad and 0.000897 mg/L for azoxystrobin. In the current study, matrix effects on LC-MS/MS detection were not investigated. Matrix-matched standards were used for quantification. In the current study, the maximum storage period from sampling to analysis was 12 days for BAS 700 F and B AS 9164 F. Residues are regarded as stable if the samples are stored deep frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95). Therefore, the stability of BAS 700 F and BAS 9164 F in BAS 736 00 F was not verified. The average recoveries for the fortification levels of 0.05 and 10.4 mg/L were found to be 96.8% and 92.0% for fluxapyroxad and 95.5% – 89.4% for azoxystrobin.

Details on measured fortification samples and obtained procedural recoveries are provided in the tables below.

**Table A 7: Procedural recoveries for BAS 736 00 F (based on measurement of BAS 700 F)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level  [mg BAS 700 F/L]** | **n** | **Mean recovery [%]** | **RSD [%]** |
| Test medium | 0.05 | 5 | 96.8 | 4 |
| Test medium | 10.4 | 5 | 92.0 | 3 |

Abbreviation: RSD = relative standard deviation

**Table A 8: Procedural recoveries for BAS 736 00 F (based on measurement of BAS 9164 F)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level  [mg BAS 9164 F/L]** | **n** | **Mean recovery [%]** | **RSD [%]** |
| Test medium | 0.05 | 5 | 95.5 | 2 |
| Test medium | 10.4 | 5 | 89.4 | 2 |

Abbreviation: RSD = relative standard deviation

**II. RESULTS AND DISCUSSION**

Analytical measurements: Analytical verification of azoxystrobin and fluxapyroxad concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of azoxystrobin ranged from 88.3% to 106% of nominal concentrations at test initiation and from 95.0% to 102% of nominal at test termination. The analyzed contents of fluxapyroxad ranged from 86.5% to 103% of nominal concentrations at test initiation and from 89.5% to 105% of nominal at test termination. The following biological results are based on nominal concentrations.

Biological results: After 48 hours of exposure no immobility occurred at nominal concentrations up to and including 1.0 mg BAS 736 00 F/L, whereas 85, 100 and 100% of the daphnids were immobile at 2.0, 4.0 and 8.0 mg BAS 736 00 F/L, respectively, which was a statistically significant increase in immobility compared to the control (Step-down Cochran-Armitage Test, one-sided greater, α = 0.05). No other sublethal effects were observed during the test. For results see Table A 9.

**Table A 9: Effects of BAS 736 00 F on *Daphnia magna* immobility**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Concentration [mg/L]  (nominal)** | **Control** | **0.5** | **1.0** | **2.0** | **4.0** | **8.0** |
| Immobility (24 h) (%) | 0 | 0 | 0 | 60\* | 90\* | 100\* |
| Immobility (48 h) (%) | 0 | 0 | 0 | 85\* | 100\* | 100\* |
|  | **Endpoints [mg BAS 736 00 F /L] (nominal)** | | | | | |
| EC50 (48 h) | 1.57 (95% confidence limit: 1.41 – 1.75) | | | | | |
| NOEC (48 h) | 1.00 | | | | | |

\* Statistically significantly different from the control (Step-down Cochran-Armitage Test, one-sided greater, α = 0.05).

|  |  |
| --- | --- |
| **Validity criteria according to OECD 202 (2004)** | **Validity criteria according to OECD 202 (2004)** |
| In the control, including the control containing the solubilizing agent, not more than 10% of the daphnids should have been immobilized. (Not more than 10% of the control daphnids should show immobilization or other signs of disease or stress, for example, discoloration or unusual behavior such as trapping at surface of water.) | 0% |
| The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. | 7.6 – 8.9 mg/L |

**III. CONCLUSION**

**In a 48-hour static acute toxicity study on *Daphnia magna,* the EC50 (48 h) of BAS 736 00 F was 1.57 mg/L based on nominal concentrations. The NOEC was determined to be 1.0 mg/L (nominal).**

* + - 1. Study 3

|  |  |
| --- | --- |
| 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.  The results refer to nominal concentrations. |

|  |  |
| --- | --- |
| Reference: | CP 10.2.1/3 |
| Report | BAS 736 00 F: Toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindák under Laboratory Conditions,  Obert-Rauser, P., 2020  report No 849661, S19-20424  2019/2039623  Authority registration No |
| Guideline(s): | OECD 201 (2006) |
| Deviations: | No |
| GLP: | yes  (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany ), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a 72-hour static toxicity laboratory study, the effect of BAS 736 00 F on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.260, 0.729, 2.04, 5.71 and 16.0 mg BAS 736 00 F/L. Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rates and yield based on number of cells.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and up to and including 5.71 mg/L. No algal cells were visible at test concentration of 16.0 mg/L. After 72 h significant inhibitory effects were determined for growth rate and yield at test item concentrations of 2.04 mg/L and above.

**In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the ErC50 for BAS 736 00 F was determined to be 4.19 mg/L and the EyC50 was 1.46 mg/L based on nominal concentrations.**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F, batch no. FD-190220-0002, content of a.s.: azoxystrobin (BAS 9164 F, reg. no. 300254: 73.9 g/L (nominal: 75.0 g/L), fluxapyroxad (BAS 700 F, reg. no. 5094351): 49.4 g/L (nominal: 50.0 g/L); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Hindàk) Korshikov (syn. *Selenastrum capricornutum*), SAG 61.81; in-house culture, stock obtained from “MBM Sciencebridge GmbH”, Göttingen, Germany.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC10, EC20 and EC50 with respect to growth rate and yield after exposure over 72 hours, NOEC.

Test concentrations: 0 (control), 0.260, 0.729, 2.04, 5.71 and 16.0 mg BAS 736 00 F/L (nominal).

Test conditions: 100 mL Erlenmeyer flasks with aluminum caps; test volume: ~50 mL; OECD 201 nutrient solution (AAP medium); pH 7.35 – 7.41 (at test initiation) and 7.98 – 8.67 (at test termination); temperature: 22.5 – 23.0 °C; initial cell densities 0.55 x 104 cells/mL; continuous light at 85.6 µEm‑2s‑1; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a LC-method with MS/MS-detection (analytical methods no. L0361/03 and L0361/01).

Statistics: Descriptive statistics; weighted non-linear regression with weighting by variability (1/Var(Y)) using a 3 parametric normal cumulative distribution function after Downhill Simplex optimization for determination of growth rate ECxx values, non-linear regression without weighting using a 3 parametric normal cumulative distribution function after Levenberg-Marquardt optimization for determination of yield ECxx values, Step-down Jonckheere-Terpstra Test Procedure for determination of the NOEC values (left sided ,α = 0.05).

**C. DESCRIPTION OF THE ANALYTICAL PRODECDURES**

Concentrations of fluxapyroxad (BAS 700 F; contained in BAS 736 00 F) and azoxystrobin (BAS 9164 F; contained in BAS 736 00 F) in test medium were determined according to the analytical methods L0361/01 and L0361/03, respectively. The validation of each analytical methods is described within separate study reports BASF DocID 2017/1065621 and 2019/1039564, respectively. Analysis of BAS 700 F and BAS 9164 F was performed by direct injection of test medium samples after dilution with acetonitrile/water/formic acid (400/600/2, v/v/v) and if necessary further dilution with diluted blank medium (test medium /(acetonitrile/water/formic acid (400/600/2, v/v/v)) (1:1, v/v)) before analysis. Final determination was accomplished by LC-MS/MS. The limit of quantification (LOQ) was 0.00119 mg/L for fluxapyroxad and 0.00178 mg/L for azoxystrobin. The limit of detection (LOD) was set to 0.0002 mg/L for fluxapyroxad and 0.0003 mg/L for azoxystrobin. In the current study, matrix effects on LC-MS/MS detection were not investigated. Matrix-matched standards were used for quantification. In the current study, the maximum storage period from sampling to analysis was 7 days for BAS 700 F and BAS 9164 F. Residues are regarded as stable if the samples are stored deep frozen for up to 30 days between sampling and analysis (EU COM 7032/VI/95). Therefore, the stability of BAS 700 F and BAS 9164 F in BAS 736 00 F was not verified. The average recoveries for the fortification levels of 0.026 and 20.8 mg/L were found to be 87.6% and 99.3% for fluxapyroxad and 89.8% – 99.2% for azoxystrobin.

Details on measured fortification samples and obtained procedural recoveries are provided in the tables below.

**Table A 10: Procedural recoveries for BAS 736 00 F (based on measurement of BAS 700 F)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level  [mg BAS 700 F/L]** | **n** | **Mean recovery [%]** | **RSD [%]** |
| Test medium | 0.026 | 5 | 87.6 | 2 |
| Test medium | 20.8 | 5 | 99.3 | 5 |

Abbreviation: RSD = relative standard deviation

**Table A 11: Procedural recoveries for BAS 736 00 F (based on measurement of BAS 9164 F)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level  [mg BAS 9164 F/L]** | **n** | **Mean recovery [%]** | **RSD [%]** |
| Test medium | 0.026 | 5 | 89.8 | 5 |
| Test medium | 20.8 | 5 | 99.2 | 3 |

Abbreviation: RSD = relative standard deviation

**II. RESULTS AND DISCUSSION**

Analytical measurements: Analytical verification of azoxystrobin and fluxapyroxad concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of azoxystrobin ranged from 98.3% to 104% of nominal concentrations at test initiation and from 97.1% to 107% of nominal at test termination. The analyzed contents of fluxapyroxad ranged from 97.5% to 103% of nominal concentrations at test initiation and from 95.3% to 107% of nominal at test termination. The following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control and up to and including 5.71 mg/L. No algal cells were visible at test concentration of 16.0 mg/L. After 72 h significant inhibitory effects were determined for growth rate and yield at test item concentrations of 2.04 mg/L and above (Step-down Jonckheere-Terpstra Test Procedure, left sided, α = 0.05). The effects on algal growth rate and yield are summarized in Table A 12.

**Table A 12: Effect of BAS 736 00 H on the growth of green alga *P. subcapitata***

| **Concentration [mg/L] (nominal)** | **0.260** | **0.729** | **2.04** | **5.71** | **16.0** |
| --- | --- | --- | --- | --- | --- |
| Inhibition in 72 h (growth rate) [%] # | -0.7 | 2.7 | 26.3\* | 61.3\* | 140.5\* |
| Inhibition in 72 h (yield) [%] # | -3.8 | 11.5 | 70.9\* | 95.1\* | 100.8\* |
|  | **Endpoints [mg BAS 736 00 H/L] (nominal)** | | | | |
| ErC50 (72 h) | 4.19 (95% confidence limits: 4.03 – 4.35) | | | | |
| ErC20 (72 h) | 1.73 (95% confidence limits: 1.54 – 1.92) | | | | |
| ErC10 (72 h) | 1.09 (95% confidence limits: 0.935 – 1.26) | | | | |
| EyC50 (72 h) | 1.46 (95% confidence limits: 1.12 – 1.90) | | | | |
| EyC20 (72 h) | 0.866 (95% confidence limits: 0.699 – 1.08) | | | | |
| EyC10 (72 h) | 0.660 (95% confidence limits: 0.526 – 0.828) | | | | |

# Negative values indicate stimulated growth

\* Statistically significant differences in growth rate and yield (Step-down Jonckheere-Terpstra Test Procedure, left sided α = 0.05) compared to the control

|  |  |
| --- | --- |
| **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. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD 201) | 102-fold |
| 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%. | 22.~~4~~% |
| The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests on *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%. | 2.0% |

**III. CONCLUSION**

**In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the ErC50 for BAS 736 00 F was determined to be 4.19 mg/L and the EyC50 was 1.46 mg/L based on nominal concentrations.**

* + 1. KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Not necessary

* + 1. KCP 10.2.3 Further testing on aquatic organisms

Not conducted

* 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 guidelines 213 and according to the principles of GLP. Validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.1.1/1 |
| Report | BAS 736 00 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions,  Wendling, K., 2019  report No 849664, S19-02328  2019/1061095  Authority registration No |
| Guideline(s): | OECD 213, OECD 214 |
| Deviations: | No |
| GLP: | yes  (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In an oral toxicity test, adult worker bees (*Apis mellifera* L.) were exposed to BAS 736 00 F. The toxicity of the test item was determined at nominal dose rates of 62.5, 125, 250, 500 and 1000 µg BAS 736 00 F/bee that is corresponding to 7.25, 14.5, 29.0, 58.0 and 116 µg total a.s./bee. The resulting oral uptake was 64.6, 114, 182, 267 and 432 µg BAS 736 00 F/bee (corresponding to 7.49, 13.2, 21.1, 31.0 and 50.1 µg total a.s./bee). Additionally, honeybees were treated with BAS 152 11 I as reference item at dose rates ranging from 0.06 to 0.14 µg dimethoate/bee (based on analyzed content of a.s.), and furthermore with a 50% (w/v) sucrose solution as control. Assessments of mortality were made after 4, 24 and 48 hours.

After 48 hours of oral exposure, a mortality of 2.5% was observed at the final assessment after 48 in the untreated control group fed with pure sucrose solution. In the test item treatment, no mortality was observed after oral consumption ≤ 182 µg consumed BAS 736 00 F/bee, after 24 and 48 hours. At the highest dose level of 432 μg consumed BAS 736 00 F/bee a maximum mortality of 25.0 (corrected 23.1%) was observed after 48 hours. Behavioral abnormalities like affected and/or apathetic and/or moribund bees were observed in the treatment groups of the nominal dose levels of 114, 182, 267 and 432 μg BAS 736 00 F/bee.

**In an acute oral toxicity test with BAS 736 00 F on honey bees, the LD50 (48 h) was determined to be > 432 µg consumed BAS 736 00 F/bee.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Honey bee (*Apis mellifera* L.) adult worker bees, derived from a healthy and queen-right colony; brushed off the combs of the honey chamber and distributed into test cages one day before test start; source: Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany.

Test design: In a 48-hour test, adult worker bees of *Apis mellifera* L. were exposed orally to BAS 736 00 F via treated food (50% (w/v) aqueous sucrose solution). The following treatment groups were set up: 5 concentration of the test item, 1 untreated control and 4 concentrations of the reference item with 6 replicates in the control and test item treatments with 4 replicates per dose rate and 10 bees per replicate, respectively. Assessments of honey bee mortality and behavioral effects were done after 4, 24 and 48 hours.

Endpoints: Mortality (LD50), behavioral impairments.

Reference item: BAS 152 11 I (a.s.: dimethoate, analyzed content: 429.0 g/L, nominal: 400 g/L).

Test concentrations: Control: untreated sucrose solution (50% w/v); test item at dose rates of 62.5, 125, 250, 500 and 1000 µg BAS 736 00 F/bee; reference item at dose rates of 0.06, 0.08, 0.11 and 0.14 µg a.s./bee.

Test item at dose rates (nominal):

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal dose [µg/bee] | | Consumed dose [µg/bee] | |
| BAS 736 00 F  [µg/bee] | Total active substance [µg/bee] | BAS 736 00 F  [µg/bee] | Total active substance [µg/bee] |
| 62.5 | 7.25 | 64.6 | 7.49 |
| 125 | 14.5 | 114 | 13.2 |
| 250 | 29.0 | 182 | 21.1 |
| 500 | 58.0 | 267 | 31.0 |
| 1000 | 116 | 432 | 50.1 |

Test conditions: Temperature: 23.7 °C - 26.0 °C; relative humidity: 53.7% - 63.2%; photoperiod: 24 h darkness (with exception of diffuse artificial light during assessments and handling, only); food: 50% (w/v) sucrose solution, *ad libitum*.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Statistical program ToxRat Professional 3.3.0 was used to analyze the study data.

**II. RESULTS AND DISCUSSION**

After 48 hours of oral exposure, a mortality of 2.5% was observed at the final assessment after 48 in the untreated control group fed with pure sucrose solution. In the test item treatment, no mortality was observed after oral consumption ≤ 182 µg consumed BAS 736 00 F/bee, after 24 and 48 hours. At the highest dose level of 432 μg consumed BAS 736 00 F/bee a maximum mortality of 25.0 (corrected 23.1%) was observed after 48 hours. Behavioral abnormalities like affected and/or apathetic and/or moribund bees were observed in the treatment groups of the nominal dose levels of 114, 182, 267 and 432 μg BAS 736 00 F/bee. The results are summarized in Table A 13.

Table A 13: Effects of BAS 736 00 F to honey bees (*Apis mellifera*) in an oral toxicity test

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **Dosage [consumed]** | **After 24 hours** | | **After 48 hours** | |
| **Mortality [%]** | | **Mortality [%]** | |
| **total** | **corr. 1)** | **total** | **corr. 1)** |
| **Control** | Sucrose solution | 0.0 | -- | 2.5 | -- |
| **BAS 736 00 F [µg/bee]** | 64.6 | 0.0 | -- | 0.0 | 0.0 |
| 114 | 0.0 | -- | 0.0 | 0.0 |
| 182 | 0.0 | -- | 0.0 | 0.0 |
| 267 | 2.5 | -- | 5.0 | 2.6 |
| 432 | 22.5 | -- | 25.0 | 23.1 |
|  | **Endpoint [µg** **consumed BAS 736 00 F/bee]** | | | | |
| LD50 (48 h) | > 432 | | | | |

1) Corrected mortality according to Abbott (1925) modified by SCHNEIDER-ORELLI (1947).

The LD50 value (24 h) for the reference item in the oral toxicity test was determined to be 0.10 µg a.s./bee (95% confidence limits: 0.09 – 0.10 µg a.s./bee) based on actual consumption.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 213 (1998)** | **Obtained in this study** |
| Control mortality ≤ 10% | 2.5% |
| LD50 (24 h) of the reference item should be in the specified range 0.10 – 0.35 µg a.s./bee | 0.10 µg a.s./bee |

All validity criteria were met.

**III. CONCLUSION**

**In an acute oral toxicity test with BAS 736 00 F on honey bees, the LD50 (48 h) was determined to be > 432 µg consumed BAS 736 00 F/bee.**

Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.1.1/2 |
| Report | BAS 700 04 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions,  Verge, E., 2014  report No EU-S14-00228,EU-704424,S14-00228  2014/1135445  Authority registration No |
| Guideline(s): | EPPO PP 1/170 (4) (2010), OECD 213, OECD 214 (1998), Van der Steen (2001) |
| Deviations: | No |
| GLP: | yes  (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In an acute oral toxicity test, young adult worker bumble bees (*Bombus terrestris*) were exposed to BAS 700 04 F. The toxicity of the test item was determined at a dose rates of 52.1, 72.9, 102.0, 142.9 and 200.0 μg a.s./bumble bee; the resulting oral uptake was 50.1, 64.1, 91.4, 121.7 and 176.3 μg a.s./bumble bee. Additionally, bumble bees were treated with BAS 152 11 I (a.s. dimethoate) as a reference item at a dose rate of 1.5 µg dimethoate/bumble bee, and furthermore with a 50% (w/v) aqueous sucrose solution as control. Assessments of bumble bee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.

After 96 hours of oral exposure, no mortality was observed in the control group. At the end of the 96-hour exposure period no mortality was recorded at the nominal dose level of 200 µg a.s./bumble bee. The maximum mortality of 3.33% was recorded at the nominal dose of 102 µg a.s./bumble bee. At the assessment after 4 hours affected and moribund bees were observed in the three highest dose levels tested. At each nominal dose of 52.1 and 102.0 µg a.s./bumble bee, one affected bee was observed at the assessment after 48 hours. At the assessments 24, 72 and 96 hours after test start no sub-lethal effects or abnormal behavior were observed.

**In the acute oral toxicity test with BAS 700 04 F, the resulting LD50 after 96 hours was estimated to be > 664.1 μg consumed BAS 700 04 F/bumble bee, corresponding to > 176.3 µg consumed a.s./bumble bee.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 700 04 F, batch No. 2122S02XS; content of a.s.: fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 27.7% (nominal 300 g/L); density: 1.130 g/cm3.

**B. STUDY DESIGN**

Test species: *Bombus terrestris* L. (bumble bee), young adult worker bumble bees derived from healthy and queen-right hives; source: Koppert, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands; collected one day before use.

Test design: In a 96-hour test, adults of *Bombus terrestris* were exposed to 5 doses of BAS 700 04 F in treated food (50% (w/v) aqueous sucrose solution). In total, 7 treatment groups were set up: 5 dose rates of the test item, 1 control group and 1 dose rate of the reference item with 3 replicates for all treatments and 10 bumble bees per replicate. Assessments of bumble bee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.

Endpoints: Mortality, behavioral impairments.

Reference item: BAS 152 11 I (dimethoate, 400.9 g/L analyzed).

Test doses: Control: 50% (w/v) aqueous sucrose solution;

BAS 700 04 F:

|  |  |  |
| --- | --- | --- |
| Nominal doses [µg a.s./bumble bee] | Actual intake [µg a.s./bumble bee] | Actual intake [µg BAS 700 04 F/bumble bee] \* |
| 52.1 | 50.1 | 188.7 |
| 72.9 | 64.1 | 241.4 |
| 102.0 | 91.4 | 344.3 |
| 142.9 | 121.7 | 458.4 |
| 200.0 | 176.3 | 664.1 |

\* calculated based on the nominal content of a.s. in BAS 700 04 F and a density of 1.130 g/cm³

Reference item: 1.5 μg dimethoate/bumble bee.

Test conditions: Temperature: 23.8°C – 26.3°C, relative humidity: 49.6% – 67.1%, photoperiod: 24 h darkness; food: 50% (w/v) aqueous sucrose solution.

Statistics: Descriptive statistics.

Analytics: Not applicable.

**II. RESULTS AND DISCUSSION**

After 96 hours of oral exposure, no mortality was observed in the control group. At the end of the 96-hour exposure period no mortality was recorded at the nominal dose level of 200 µg a.s./bumble bee. The maximum mortality of 3.33% was recorded at the nominal dose of 102 µg a.s./bumble bee. At the assessment after 4 hours affected and moribund bees were observed in the three highest dose levels tested. At each nominal dose of 52.1 and 102.0 µg a.s./bumble bee, one affected bee was observed at the assessment after 48 hours. At the assessments 24, 72 and 96 hours after test start no sub-lethal effects or abnormal behavior were observed. The results are summarized below.

Table A 14: Toxicity of BAS 700 04 F to *Bombus terrestris* (bumble bee) in an oral toxicity test

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment [µg a.s./bumble bee]** | **Intake [µg a.s./bumble bee]** | **Mortality [%]** | | | |
| **24 h** | **48 h** | **72 h** | **96 h** |
| Control | -- | 0.0 | 0.0 | 0.0 | 0.0 |
| 52.1 | 50.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 72.9 | 64.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 102.0 | 91.4 | 3.3 | 3.3 | 3.3 | 3.3 |
| 142.9 | 121.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| 200.0 | 176.3 | 0.0 | 0.0 | 0.0 | 0.0 |
|  | **Endpoints** | | | | |
| LD50 (96 h) | **[µg consumed a.s./bumble bee]** | | **[µg consumed BAS 700 04 F/bumble bee]** | | |
| > 176.3 | | > 664.1 | | |

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 247 (2017)** | **Obtained in this study** |
| Control mortality ≤ 10% | 0% |
| Reference item mortality ≥ 50% | 73.3% (at 1.5 µg a.s./bumble bee) |

All validity criteria were met.

**III. CONCLUSION**

**In the acute oral toxicity test with BAS 700 04 F, the resulting LD50 after 96 hours was estimated to be > 664.1 μg consumed BAS 700 04 F/bumble bee, corresponding to > 176.3 µg consumed a.s./bumble bee.**

* + - * 1. KCP 10.3.1.1.2 Acute contact toxicity to bees

Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | The study was conducted to OECD guidelines 214 and according to the principles of GLP. Validity criteria were met.  The study is considered to be reliable and suitable for the risk assessment. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.1.2/1 |
| Report | BAS 736 00 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions,  Wendling, K., 2019  report No 849664, S19-02328  2019/1061095  Authority registration No |
| Guideline(s): | OECD 213, OECD 214 |
| Deviations: | No |
| GLP: | yes  (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a contact toxicity test, adult worker bees (*Apis mellifera* L.) were exposed to BAS 736 00 F. The toxicity of the test item was determined at nominal dose rates of 62.5, 125, 250, 500 and 1000 µg BAS 736 00 F/bee that is corresponding to 7.25, 14.5, 29.0, 58.0 and 116 µg total a.s./bee. Additionally, honey bees were treated with BAS 152 11 I as reference item at dose rates ranging from 0.10 to 0.34 µg a.s./bee (based on analyzed content of a.s.: dimethoate), and furthermore with Triton X-100 solution as control. Assessments of mortality and behavioral abnormalities were made after 4, 24, 48 and 72 hours.

After 72 hours of contact exposure, a mortality of 2.5% occurred in the control group. The maximum mortality observed in the contact toxicity test with BAS 736 00 F was 25.0% (corrected 23.1%) at the test concentration of 500 µg BAS 736 00 F /bee after 72 hours. Behavioral abnormalities like affected and/or apathetic and/or moribund bees were observed at all treatment groups.

**In an acute contact toxicity test with BAS 736 00 F on honey bees, the LD50 (48 h) was determined to be > 1000 µg BAS 736 00 F/bee.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Honey bee (*Apis mellifera* L.) adult worker bees, derived from a healthy and queen-right colony; brushed off the combs of the honey chamber and distributed into test cages one day before test start; source: Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany.

Test design: In a 72-hour test, adult worker bees of *Apis mellifera* L. were exposed to BAS 736 00 F in an appropriate carrier (0.1% Triton X-100 as wetting agent) placed on the dorsal bee thorax. The following treatment groups were set up: 5 concentration of the test item, 2 untreated control and 4 concentrations of the reference item with 4 replicates for all treatments and 10 bees per replicate, respectively. Assessments of honey bee mortality and behavioral effects were done after 4, 24, 48 and 72 hours.

Endpoints: Mortality (LD50), behavioral impairments.

Reference item: BAS 152 11 I (a.s.: dimethoate, analyzed content: 429.0 g/L, nominal: 400 g/L).

Test concentrations: Solvent control: deionized water + 0.1% (v/v) Triton X-100; test item at dose rates of 62.5, 125, 250, 500 and 1000 µg BAS 736 00 F/bee; reference item at dose rates of 0.10, 0.15, 0.23 and 0.34 µg a.s./bee.

Test item at dose rates (nominal):

|  |  |
| --- | --- |
| Nominal dose [µg/bee] | |
| BAS 736 00 F [µg/bee] | Total active substance [µg/bee] |
| 62.5 | 7.25 |
| 125 | 14.5 |
| 250 | 29.0 |
| 500 | 58.0 |
| 1000 | 116 |

Test conditions: Temperature: 23.7 °C - 26.0 °C; relative humidity: 53.7% - 63.2%; photoperiod: 24 h darkness (with exception of diffuse artificial light during assessments and handling, only); food: 50% (w/v) sucrose solution, *ad libitum*.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Statistical program ToxRat Professional 3.3.0 was used to analyze the study data.

**II. RESULTS AND DISCUSSION**

After 72 hours of contact exposure, a mortality of 2.5% occurred in the control group. The maximum mortality observed in the contact toxicity test with BAS 736 00 F was 25.0% (corrected 23.1%) at the test concentration of 500 µg BAS 736 00 F /bee after 72 hours. Behavioral abnormalities like affected and/or apathetic and/or moribund bees were observed at all treatment groups. The results are summarized in Table A 15.

**Table A 15: Effects of BAS 736 00 F to honey bees (*Apis mellifera*) in a contact toxicity test**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Dosage [applied]** | **After 24 hours** | | **After 48 hours** | | **After 72 hours** | |
| **Mortality [%]** | | **Mortality [%]** | | **Mortality [%]** | |
| **total** | **corr. 1)** | **total** | **corr. 1)** | **total** | **corr. 1)** |
| **Control** | Water + Triton X-100 | 2.5 | -- | 2.5 | -- | 2.5 | -- |
| **BAS 736 00 F [µg/bee]** | 62.5 | 0.0 | -2.6 | 0.0 | -2.6 | 5.0 | 2.6 |
| 125 | 2.5 | 0.0 | 7.5 | 5.1 | 12.5 | 10.3 |
| 250 | 5.0 | 2.6 | 12.5 | 10.3 | 20.0 | 17.9 |
| 500 | 0.0 | -2.6 | 15.0 | 12.8 | 25.0 | 23.1 |
| 1000 | 0.0 | -2.6 | 5.0 | 2.6 | 7.5 | 5.1 |
|  | **Endpoint [µg/bee]** | | | | | | |
| LD50 (72 h) | > 1000 | | | | | | |

1) Corrected mortality according to Abbott (1925) modified by SCHNEIDER-ORELLI (1947). Negative values indicate lower mortality compared to the control group.

The LD50 value (24 h) for the reference item in the contact toxicity test was determined to be 0.22 µg a.s./bee (95% confidence limits: 0.20 – 0.25 µg a.s./bee).

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 214 (1998)** | **Obtained in this study** |
| Control mortality ≤ 10% | 2.5% control |
| LD50 (24 h) of the reference item should be in the specified range 0.10 – 0.30 µg a.s./bee | 0.22 µg a.s./bee |

All validity criteria were met.

**III. CONCLUSION**

**In an acute contact toxicity test with BAS 736 00 F on honey bees, the LD50 (48 h) was determined to be > 1000 µg BAS 736 00 F/bee.**

Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.1.2/2 |
| Report | BAS 700 04 F - Acute oral and contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions,  Verge, E., 2014  report No EU-S14-00228,EU-704424,S14-00228  2014/1135445  Authority registration No |
| Guideline(s): | EPPO PP 1/170 (4) (2010), OECD 213, OECD 214 (1998), Van der Steen (2001) |
| Deviations: | No |
| GLP: | yes  (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In an acute contact toxicity test, young adult worker bumble bees (*Bombus terrestris*) were exposed to BAS 700 04 F. a dose rates of 52.1, 72.9, 102.0, 142.9 and 200.0 μg a.s./bumble bee; the resulting oral uptake was 196.2, 274.6, 384.2, 538.3 and 753.3 μg BAS 700 04 F/bumble bee. Additionally, bumble bees were treated with BAS 152 11 I (a.s. dimethoate) as a reference item at a dose rate of 12 µg dimethoate/bumble bee, and furthermore with tap water as control. Assessments of bumble bee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.

After 96 hours of contact exposure, no mortality was observed in the control. After 96 hours of exposure no mortality was observed in the BAS 700 04 F treatment groups. No sub-lethal effects or abnormal behavior were observed during the entire 96-h test period.

**In the acute contact toxicity test with BAS 700 04 F, the resulting LD50 after 96 hours was estimated to be > 753.3 μg BAS 700 04 F/bumble bee, corresponding to > 200 µg a.s./bumble bee.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 700 04 F, batch No. 2122S02XS; content of a.s.: fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 27.7% (nominal 300 g/L); density: 1.130 g/cm3.

**B. STUDY DESIGN**

Test species: *Bombus terrestris* L. (bumble bee), young adult worker bumble bees derived from healthy and queen-right hives; source: Koppert, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands; collected one day before use.

Test design: In a 96 hour test, adults of *Bombus terrestris* were exposed to 5 dose rates of BAS 700 04 F placed on the dorsal bumble bee thorax. In total, 7 treatment groups were set up: 5 dose rate of the test item, 1 control group and 1 dose rate of the reference item with 3 per treatment and 10 bumble bee per replicate. Assessments of bumble bee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.

Endpoints: Mortality, behavioral impairments.

Reference item: BAS 152 11 I (dimethoate, 400.9 g/L analyzed).

Test doses: Control: tap water,

BAS 500 F:

|  |  |
| --- | --- |
| Nominal doses [µg a.s./bumble bee] | Nominal doses [µg BAS 700 04 F/bumble bee] \* |
| 52.1 | 196.2 |
| 72.9 | 274.6 |
| 102.0 | 384.2 |
| 142.9 | 538.3 |
| 200.0 | 753.3 |

\* calculated based on the nominal content of a.s. in BAS 700 04 F and a density of 1.130 g/cm³

Reference item: 12 μg dimethoate/bumble bee.

Test conditions: Temperature: 23.7°C – 26.1°C, relative humidity: 53.1% – 64.6%, photoperiod: 24 h darkness; food: 50% (w/v) aqueous sucrose solution.

Statistics: Descriptive statistics.

Analytics: Not applicable.

**II. RESULTS AND DISCUSSION**

After 96 hours of contact exposure, no mortality was observed in the control. After 96 hours of exposure no mortality was observed in the BAS 700 04 F treatment groups. No sub-lethal effects or abnormal behavior were observed during the entire 96-h test period. The results are summarized below.

Table A 16: Toxicity of BAS 700 04 F to *Bombus terrestris* (bumble bee) in a contact toxicity test

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment [µg a.s./bumble bee]** | **Mortality [%]** | | | |
| **24 h** | **48 h** | **72 h** | **96 h** |
| **Control** | 0.0 | 0.0 | 0.0 | 0.0 |
| 52.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 72.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| 102.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 142.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| 200.0 | 0.0 | 0.0 | 0.0 | 0.0 |
|  | **Endpoints** | | | |
| LD50 (96 h) | **[µg consumed a.s./bumble bee]** | | **[µg consumed BAS 700 04 F/bumble bee]** | |
| > 200 | | > 753.3 | |

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 246 (2017)** | **Obtained in this study** |
| Control mortality ≤ 10% | 0% |
| Reference item mortality ≥ 50% | 83.3% at 12 µg a.s./bumble bee |

All validity criteria were met.

**III. CONCLUSION**

**In the acute contact toxicity test with BAS 700 04 F, the resulting LD50 after 96 hours was estimated to be > 753.3 μg BAS 700 04 F/bumble bee, corresponding to > 200 µg a.s./bumble bee.**

* + - 1. KCP 10.3.1.2. Chronic toxicity to bees
         1. Study 1

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.2/1 |
| Report | Chronic toxicity of BAS 700 F tested as BAS 700 04 F to the honey bee Apis mellifera L. under laboratory conditions,  Dressler, K., 2021  report No 863261, 2048BAC0072  2020/2083866  Authority registration No |
| Guideline(s): | OECD 245 (2017) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a 10-day chronic toxicity feeding test, max. 2-day old worker honey bees (*Apis mellifera* L. ssp. Buckfast) were exposed to a daily application of BAS 700 04 F diluted in the bee food. The chronic oral toxicity of the test item was determined at nominal doses of 47.1, 94.1, 188, 377 and 753 µg product/bee/day (effective doses were 46.5, 108, 172, 413 and 697 μg product/bee/day), corresponding to concentrations of 1.199, 2.397, 4.794, 9.588 and 19.176 g product/kg food. Additionally, honey bees were treated with Danadim® Progress (dimethoate) as reference item at a nominal dose of 27.3 ng a.s./bee/day. Untreated diet was served as a control and untreated diet containing 0.1% (w/v) xanthan served as viscosifier control. Assessments of mortality, food consumption and behavioral abnormalities were done daily.

After 10 days of continuous exposure, a mean mortality of 3.3% was observed in both control groups. In the test item group, bees effectively consumed doses of 46.5, 108, 172, 413 and 697 µg BAS 700 04 F/bee/day which resulted in mortalities of 0.0, 0.0, 0.0, 3.3 and 6.7%, respectively. The resulting corrected mortalities were 0.0, 0.0, 0.0, 0.0 and 3.4%. None of the obtained mortalities were statistically significantly increased compared to the viscosifier control group. No behavioral abnormalities were observed in any test item treatment group on any assessment day.

**In a 10-day chronic toxicity feeding test with BAS 700 04 F the NOEDD and NOEC were determined to be ≥ 697 μg consumed product/bee/day and ≥ 19.176 mg product/kg food, respectively. The LDD50 and LC50 were determined to be > 697 μg consumed product/bee/day and > 19.176 mg product/kg food, respectively.**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 700 04 F; batch no. FRE-001683; content of a.s.: fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 300.8 g/L analyzed (300.0 g/L nominal); density: 1.130 g/cm³.

**B. STUDY DESIGN**

Test species: *Apis mellifera* L. ssp. Buckfast (honey bee), max. 2-day old bees; derived from healthy and queen-right colonies; source: in-house colonies.

Test design: In a 10-day chronic test, young adults of *Apis mellifera* L. were daily exposed to 5 doses of BAS 700 04 F in treated food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The following treatment groups were set up: 5 doses of the test item, one untreated control group fed with 50% (w/v) aqueous sucrose solution, one viscosifier control group fed with 50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan and one dose of the reference item with 3 replicates per dose and 10 bees per replicate. Assessments of bee mortality, food consumption and behavioral effects were done daily during the study. Concentration of both active substances in the highest and lowest test item feeding concentration were determined analytically on the first and last day of application.

Endpoint: Mortality (LDD50, LC50, NOEDD, NOEC), behavioral abnormalities.

Reference item: Danadim® Progress (411.2 g dimethoate/L analysed, 400 g/L nominal).

Test concentrations: Control (50% (w/v) aqueous sucrose solution);

Viscosifier control (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan);

test item (applied in 50% (w/v) sucrose solution + 0.1% (w/v) xanthan):

|  |  |  |  |
| --- | --- | --- | --- |
| Nominal dose [µg/bee/day] | | Consumed dose [µg/bee/day] | |
| BAS 700 04 F | BAS 700 F | BAS 700 04 F | BAS 700 F |
| 47.1 | 12.5 | 46.5 | 12.3 |
| 94.1 | 25.0 | 108 | 28.5 |
| 188 | 50.0 | 172 | 45.7 |
| 377 | 100 | 413 | 110 |
| 753 | 200 | 697 | 185 |

The doses correspond to concentrations of 1.199, 2.397, 4.794, 9.588 and 19.176 g product/kg food (equivalent to 0.318, 0.636, 1.273, 2.546 and 5.091 g BAS 700 F/kg food).

Reference item (applied in 50% sucrose solution): treated diet at a nominal dose of 27.3 ng dimethoate/bee/day (corresponding to a concentration of 0.694 mg dimethoate/kg food).

Test conditions: Temperature: 31.9 – 33.4 °C, relative humidity: 56.3 – 63.5%, photoperiod: constant darkness (diffuse artificial light during assessments), food: 50% (w/v) aqueous sucrose solution.

Analytics: Analytical verification of the test item was conducted by BASF method L0372/02 using high performance liquid chromatography (HPLC) with mass-spectrometric (MS-MS) detection.

Statistics: Descriptive statistics; Fisher’s Exact Binomial Test with Bonferroni Correction for comparison of control with test item treatment group and determination of NOEDD and NOEC (one-sided greater, α = 0.05). Due to the lack of effects in all dose groups, the LDDX and LCX values could not be calculated. They were estimated from the generated raw data

**C. DESCRIPTION OF THE ANALYTICAL PROCEDURES**

Concentrations of BAS 700 F (contained in BAS 700 04 F) in honey bee feeding solution were determined according to the analytical method L0372/02. The validation of the analytical method is described in the study report. A 0.2 g aliquot of sample was extracted with 4 mL of methanol/water 75/25 (v/v). The mixture was shaken for 30 minutes and centrifuged. The extract was transferred into a 10 mL-measuring flask and filled to the mark with methanol/water 75/25 (v/v). 1 mL-aliquot was added to a 2 mL-centrifugation tube containing 150 mg MgSO4, 50 mg C18-EC and 50 mg PSA. The tube was shaken for 30 s on a vortexer and 400 μl of the extract diluted with 600 μl methanol/water 75/25 (v/v). If necessary, the diluted extracts were further diluted into the range of the calibration curve before injecting into the HPLC system. The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was set to ≤ 30% of LOQ (0.00188 mg/kg). Matrix effects were taken into account by the addition of the same amount of blank extract to calibration samples as included in the analysis samples. Thus, all measured samples contained the same amount of original sample matrix. Since the samples of the test item feeding solutions were analyzed within 30 days, the analysis of storage stability samples did not take place. Details on measured fortification samples and obtained procedural recoveries for BAS 700 F are given in Table A 17.

Table A 17: Procedural recoveries for BAS 700 F in honey bee diet

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Substance** | **Matrix** | **Fortification level (mg/kg)** | **n** | **Mean (%)** | **RSD (%)** |
| BAS 700 F | Honey bee diet | 0.010 | 4\* | 89.7 | 13.5 |
| 6711 | 5 | 94.4 | 6.54 |

\*One out of five samples was out of range confirmed by Dixon test

**II. RESULTS AND DISCUSSION**

After 10 days of continuous exposure, a mean mortality of 3.3% was observed in both control groups. In the test item group, bees effectively consumed doses of 46.5, 108, 172, 413 and 697 µg BAS 700 04 F/bee/day which resulted in mortalities of 0.0, 0.0, 0.0, 3.3 and 6.7%, respectively. The resulting corrected mortalities were 0.0, 0.0, 0.0, 0.0 and 3.4%. None of the obtained mortalities were statistically significantly increased compared to the viscosifier control group (Fisher’s Exact Binomial Test with Bonferroni Correction, α = 0.05, one-sided greater). No behavioral abnormalities were observed in any test item treatment group on any assessment day. The results are summarized in Table A 18.

Table A 18: Toxicity of BAS 700 04 F to *Apis mellifera* L. in a chronic toxicity feeding test after

10 days

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | | | **Mortality after 10 days** | | | |
| **Nominal dosage** [µg product/bee/day] | **Consumed dosage** [µg product/bee/day] | **Concentration** [mg product/kg food] | **Mean mortality** [%] | | **Corrected mortality** [%] | **Number of bees with behavioral abnormalities 1)** |
| Control | -- | -- | 3.3 | | -- | 0 (29) |
| Viscosifier control | -- | -- | 3.3 | | -- | 0 (29) |
| 47.1 | 46.5 | 1.199 | 0.0 | | 0.0 | 0 (30) |
| 94.1 | 108 | 2.397 | 0.0 | | 0.0 | 0 (30) |
| 188 | 172 | 4.794 | 0.0 | | 0.0 | 0 (30) |
| 377 | 413 | 9.588 | 3.3 | | 0.0 | 0 (29) |
| 753 | 697 | 19.176 | 6.7 | | 3.4 | 0 (28) |
| **Endpoints [10 days]** | | | | | | |
| Test item doses | LDD50/20/10 [μg consumed product/bee/day] 2) 3) | | | > 697 | | |
| LDD50/20/10 [μg consumed a.s./bee/day] 2) 3) | | | > 185 | | |
| NOEDD [μg consumed product/bee/day] 2) 4) | | | ≥ 697 | | |
| NOEDD [μg consumed a.s./bee/day] 2) 4) | | | ≥ 185 | | |
| Test item concentrations | LC50/20/10 [g product/kg food]3) | | | > 19.176 | | |
| LC50/20/10 [g a.s./kg food] 3) | | | > 5.091 | | |
| NOEC [g product/kg food]4) | | | ≥ 19.176 | | |
| NOEC [g a.s./kg food]4) | | | ≥ 5.091 | | |

Corrected mortality (according to SCHNEIDER-ORELLI 1947); Mortality of the test item treatment group was corrected for mortality of untreated viscosifier control group.

1) Behavioral abnormalities at the end of the test referring to the number of remaining bees, given in parentheses.

2) Taking into account the actual food uptake and evaporation.

3) Lethal dietary doses/concentrations were estimated from the generated raw data.

4) No observed effect dietary dose/concentration were calculated using Fisher’s Exact Binomial Test with Bonferroni Correction (α = 0.05; one-sided greater).

The reference item, applied at 27.3 ng a.s./bee/day (equivalent to 0.696 mg a.s./kg food), caused 100% mortality of the exposed honey bees after 10 days.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 245 (2017)** | **Obtained in this study** |
| Control mortality from ≤ 15% at D10 across all replicates | 3.3% both controls |
| Reference item mortality ≥ 50% on D10 | 100% |

All validity criteria were met.

**III. CONCLUSION**

**In a 10-day chronic toxicity feeding test with BAS 700 04 F the NOEDD and NOEC were determined to be ≥ 697μg consumed product/bee/day and ≥ 19.176 mg product/kg food, respectively. The LDD50 and LC50 were determined to be > 697μg consumed product/bee/day and > 19.176 mg product/kg food, respectively.**

* + - * 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.2/2 |
| Report | BAS 736 00 F - Honey Bee (Apis mellifera L.) Chronic Oral Toxicity Test - 10 Day Feeding Test in the Laboratory,  Haeuser, R., 2021  report No 900786, S20-05931  2020/2080999  Authority registration No |
| Guideline(s): | OECD 245 (2017) |
| Deviations: | No |
| GLP: | yes  (certified by LUBW Landesanstalt fuer Umwelt Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a 10-day chronic toxicity feeding test max. 2-day old worker honey bees (*Apis mellifera* L.) were exposed to a daily application of BAS 736 00 F diluted in bee food. The chronic toxicity of the test item was determined at nominal doses of 25.0, 50.0, 100, 200 and 400 µg product/bee/day (effective doses were 19.2, 28.3, 40.7, 60.2 and 92.2 µg product/bee/day), corresponding to concentrations of 0.637, 1.27, 2.55, 5.09 and 10.2 g product/kg food. Additionally, honey bees were treated with BAS 152 65 I (dimethoate) as reference item at a nominal dose of 0.02 µg a.s./bee/day. Untreated diet was served as a control. Assessments of mortality and behavioral abnormalities were done daily.

After 10 days of continuous exposure, no mortality was observed in the control group. In the test item groups, bees effectively consumed doses of 19.2, 28.3, 40.7, 60.2 and 92.2 µg BAS 736 00 F/bee/day which resulted in cumulative mortalities of 0.0, 35.0, 57.5, 100.0 and 100.0%, respectively. A statistically significant increase in mortality was found in the test item groups with an actual consumed dose of 28.3, 40.7, 60.2 and 92.2 µg BAS 736 00 F/bee/day compared to the control group. Behavioral abnormalities were observed in a few bees in terms of affected or moribund, at the concentrations of 0.637, 1.27, 2.55, 5.09 and 10.2 g BAS 736 00 F/kg food at several assessment intervals during the 10-day observation period.

**In a 10-day chronic toxicity feeding test with BAS 736 00 F the NOEDD and NOEC were determined to be 19.2 μg consumed product/bee/day and 0.637 g product/kg food, respectively. The LDD50 and LC50 were determined to be 36.6 μg consumed product/bee/day and 1.89 g product/kg food, respectively.**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Honey bee (*Apis mellifera* L.), max. 2-day old bees; derived from healthy and queen-right colonies; source: test facility own stock.

Test design: In a 10-day chronic test, young adults of *Apis mellifera* L. were exposed daily to 5 doses of BAS 736 00 F in treated food (50% (w/v) aqueous sucrose solution). The following treatment groups were set up: 5 doses of the test item, one untreated control group fed with 50% (w/v) aqueous sucrose solution and one dose of the reference item with 4 replicates per dose and 10 bees per replicate. Assessments of bee mortality, food consumption and behavioral effects were done daily during the study.

Endpoint: Mortality (LDD10/20/50, LC10/20/50, NOEDD, NOEC, LOEDD, LOEC), behavioral abnormalities.

Reference item: BAS 152 65 I (a.s.: dimethoate, analyzed content: 412 g/L, nominal: 400 g/L).

Test concentrations: Control: 50% (w/v) aqueous sucrose solution;

Test item (applied in 50% (w/v) sucrose solution):

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Nominal dose [µg/bee/day] | | | | Consumed dose [µg/bee/day] | | | |
| BAS 736 00 F | BAS 9164 F | BAS 700 F | BAS 736 00 F | | BAS 9164 F | BAS 700 F |
| 25.0 | 1.74 | 1.16 | 19.2 | | 1.34 | 0.891 |
| 50.0 | 3.48 | 2.32 | 28.3 | | 1.97 | 1.31 |
| 100 | 6.96 | 4.64 | 40.7 | | 2.83 | 1.89 |
| 200 | 13.9 | 9.28 | 60.2 | | 4.19 | 2.79 |
| 400 | 27.8 | 18.6 | 92.2 | | 6.42 | 4.28 |

The doses correspond to concentrations of 0.637, 1.27, 2.55, 5.09 and 10.2 g BAS 736 00 F/kg food (equivalent to 0.0443, 0.0884, 0.177, 0.354 and 0.710 mg BAS 9164 F/kg food and 0.0296, 0.0589, 0.118, 0.236 and 0.473 mg BAS 700 F/kg food).

Reference item (applied in 50% sucrose solution): treated diet at a dose rate of 0.02 µg consumed dimethoate/bee/day (corresponding to a concentration of 0.9 mg dimethoate/kg food, based on the analyzed content of the a.s).

Test conditions: Temperature: 32.1°C to 32.6°C (acclimatization), 32.0°C to 32.8°C (exposure), relative humidity: 52.5% to 61.6% (acclimatization), 51.4% to 64.1% (exposure), photoperiod: constant darkness except during application, food: 50% (w/v) aqueous sucrose solution.

Analytics: Analytical verification of the test item was conducted by BASF method L0372/02 using liquid chromatography with mass-spectrometric (LC-MS/MS) detection.

Statistics: Descriptive statistics; Cochran-Armitage test (one-sided greater, α = 0.05) for NOEC/NOEDD determination; Probit analysis using linear max. likelihood regression for LC10,20,50 determination; Weibull analysis using linear max. likelihood regression for LDD10,20,50 determination.

**C. DESCRIPTION OF THE ANALYTICAL PROCEDURES**

Concentration control analysis of BAS 736 00 F via its representative active ingredients BAS 700 F in honey bee feeding solution was determined according to the analytical method L0372/02. The validation of the analytical method is described in the study report. 0.840 mL of the thawed and vortexed samples were transferred into a 50 mL plastic tube, then filled up to the mark with methanol/water (3:1, v/v). The mixture was shaken for 30 s. 1 mL of the extract was transferred into a PSA-Kit-3a and then shaken again for another 30 s. The samples were centrifuged at maximum rpm for 5 min and then diluted with methanol/water (3:1, v/v). If necessary, the diluted extracts were further diluted into the range of the calibration curve before injecting into the LC-MS/MS system. The determination was performed by LC-MS/MS. The limit of quantification (LOQ) was 0.0102 mg/kg and the limit of detection (LOD) is 0.0025 mg/kg. Matrix effects were compensated by using matrix-matched calibration standards with solvent standards (= standards in methanol/water (3:1, v/v)) at identical nominal concentrations. The effects were assessed by comparing the standard’s peak areas. Since the samples of the test item feeding solutions were analyzed within 29 days, the analysis of storage stability samples did not take place. Details on measured fortification samples and obtained procedural recoveries for BAS 700 F are given in Table A 19.

**Table A 19: Procedural recoveries for BAS 700 F in honey bee food**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Substance** | **Matrix** | **Test item Fortification level (mg/kg)** | **Fortification level Fluxapyroxad (mg/kg)** | **n\*** | **Mean (%)** | **RSD (%)** |
| BAS 700 F | Honey bee food | 0.219 | 0.0102 | 5 | 100 | 11 |
| 13100 | 608 | 4 | 107 | 1.4 |

*RSD = relative standard deviation*

\* One outlier according to Grubbs, not used for calculation of mean and RSD.

**II. RESULTS AND DISCUSSION**

After 10 days of continuous exposure, no mortality was observed in the control group. In the test item groups, bees effectively consumed doses of 19.2, 28.3, 40.7, 60.2 and 92.2 µg BAS 736 00 F/bee/day which resulted in cumulative mortalities of 0.0, 35.0, 57.5, 100.0 and 100.0%, respectively. A statistically significant increase in mortality was found in the test item groups with an actual consumed dose of 28.3, 40.7, 60.2 and 92.2 µg product/bee/day compared to the control group (Cochran-Armitage test, α = 0.05, one-sided greater). Behavioral abnormalities were observed in a few bees in terms of affected or moribund, at the concentrations of 0.637, 1.27, 2.55, 5.09 and 10.2 g BAS 736 00 F/kg food at several assessment intervals during the 10-day observation period.

The results are summarized in Table A 20.

Table A 20: Toxicity of BAS 736 00 F to *Apis mellifera* L. in a chronic toxicity feeding test after

10 days

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | | | **Mortality after 10 days** | | |
| **Nominal dosage** [µg product/bee/day] | **Consumed dosage** [µg product/bee/day] | **Concentration** [g product/kg food] | **Mean cumulative mortality** [%] | | **Number of bees with behavioral abnormalities 1)** |
| Control | -- | -- | 0.0 | | 0 (40) |
| 25.0 | 19.2 | 0.637 | 0.0 | | 1 (40) |
| 50.0 | 28.3 | 1.27 | 35.0\* | | 2 (40) |
| 100 | 40.7 | 2.55 | 57.5\* | | 3 (39) |
| 200 | 60.2 | 5.09 | 100.0\* | | 6 (34) |
| 400 | 92.2 | 10.2 | 100.0\* | | 6 (16) |
| **Endpoints [10 days]** | | | | | |
| Test item doses | LDD10 [μg consumed product/bee/day] 2) 3) | | | 22.8 | |
| LDD20 [μg consumed product/bee/day] 2) 3) | | | 27.5 | |
| LDD50 [μg consumed product/bee/day] 2) 3) | | | 36.6 | |
| NOEDD [μg consumed product/bee/day] 2) 4) | | | 19.2 | |
| LOEDD [μg consumed product/bee/day] 2) 4) | | | 28.3 | |
| Test item concentration | LC10 [g product/kg food] 3) | | | 0.937 | |
| LC20 [g product/kg food] 3) | | | 1.19 | |
| LC50 [g product/kg food] 3) | | | 1.89 | |
| NOEC [g product/kg food] 4) | | | 0.637 | |
| LOEC [g product/kg food] 4) | | | 1.27 | |

\* Statistically significantly different compared to the control (Cochran-Armitage test, α = 0.05, one-sided greater).

1) Behavioral abnormalities (affected and moribund) at the end of the test referring to the number of remaining bees, given in parentheses.

2) Based on the actual food uptake and evaporation.

3) Calculated using Probit analysis using linear max. likelihood regression; lack of fit significant with a p(Chi²) = 0.03

4) Calculated using Cochran-Armitage test, α = 0.05; one-sided greater).

The reference item, applied at 0.02 µg a.s./bee/day (equivalent to 0.9 mg a.s./kg food), caused 82.5% mortality of the exposed honeybees after 10 days.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 245 (2017)** | **Obtained in this study** |
| Control mortality from ≤ 15% at D10 across all replicates | 0.0% |
| Reference item mortality ≥ 50% on D10 | 82.5% |

All validity criteria were met.

**III. CONCLUSION**

**In a 10-day chronic toxicity feeding test with BAS 736 00 F the NOEDD and NOEC were determined to be 19.2 μg consumed product/bee/day and 0.637 g product/kg food, respectively. The LDD50 and LC50 were determined to be 36.6 μg consumed product/bee/day and 1.89 g product/kg food, respectively.**

* + - 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.3/1 |
| Report | Repeated exposure of honey bee (Apis mellifera) larvae to BAS 700 F (Fluxapyroxad) under laboratory conditions (in vitro),  Kleebaum, K., 2018  report No 726242, 1748BLC0043  2017/1036679  Authority registration No |
| Guideline(s): | OECD 239 (2016) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of the test item BAS 700 F (Reg. No. 5 094 351) on survival and adult emergence of honey bee larvae (*Apis mellifera*)were investigated in a laboratory test with repeated exposure over a time period of 22 days. Synchronized 1st larval stage (L1) honey bee larvae (*Apis mellifera*) were fed with artificial diet for 5 days (day 1, 3, 4, 5 and 6). On days 3, 4, 5 and 6, larvae were fed with diet containing five different concentrations of BAS 700 F (Reg. No. 5 094 351) resulting in concentrations of 32, 63, 126, 253 and 506 mg a.s./kg food, corresponding to total doses of 5.0, 10, 20, 40 and 80 μg a.s./larva. Untreated diet served as a control, in addition to a solvent control with acetone equivalent to the dose used in the treatment groups. Furthermore, dimethoate at a dose rate of 7.6 µg/larva served as reference item treatment. All treatment groups and controls contained larvae from three different bee colonies. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment. Additionally, other observations such as small body size or large quantities of remaining food after 120 hours were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

After 120 hours of repeated oral exposure (on D8) larval mortalities of 0.0 and 2.8% were observed in the controls, respectively. Pupal mortality (between D8 and D22) was 19.4% in the untreated control and 17.1% in the solvent control. The control groups showed a total mortality of 19.4% in the untreated and in the solvent control, respectively, at D22. In the test item group, larval mortalities at D8 ranged between 0.0 and 2.8%. Pupal mortalities ranged between 14.3 and 41.7% in the test item treatment groups. Total mortalities at D22 ranged between 16.7 and 41.7%. On D8, no statistically significantly increased mortality occurred in any of the larvae groups after being treated with BAS 700 F and none individual of all remaining larvae showed remaining food. In the final assessment at D22, adult emergence rates of 80.6% were determined for the honey bees in both control groups. In the test item group, the adult honey bees emerged at rates ranging between 58.3 and 83.3% following an application of 5.0, 10, 20, 40 and 80 μg a.s./larva, respectively, during the larval stages. No statistically significantly increased mortality occurred in any of the larvae groups after being treated with BAS 700 F.

**In a repeated exposure larval toxicity study with BAS 700 F, the LD50 (larval mortality on D8) was estimated to be > 80 μg a.s./larva, which is equivalent to a LC50 of > 506 mg a.s./kg food. The respective NOED was ≥ 80 μg a.s./larva and the corresponding NOEC was ≥ 506 mg a.s./kg food. The ED50 (successful adult emergence up to D22) was estimated to be > 80 μg a.s./larva, which is equivalent to an EC50 of > 506 mg a.s./kg food. The respective NOED was ≥ 80 μg a.s./larva and the corresponding NOEC was ≥ 506 mg a.s./kg food.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 700 F (fluxapyroxad, Reg. No. 5 094 351); batch no.: COD-001466; analyzed purity: 100.0% ± 1%.

**B. STUDY DESIGN**

Test species: Larvae of *Apis mellifera* L. subspeciesBuckfast (honey bee); synchronized first larval stage (L1); derived from at least three healthy and queen-right colonies; source: BioChem agrar, Germany.

Test design: 22-day repeated exposure larval toxicity test according to OECD 239 (2016). L1 honey bee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment (on D1). After this, the larvae were fed during larval development with artificial diet, containing the test item on rearing days 3, 4, 5 and 6. In total, 8 treatment groups were set up: 5 doses of the test item, 1 untreated control group, 1 solvent control and 1 dose of the reference item, each with 3 replicates and 12 larvae per replicate. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of remaining food after 120 hours (D8) were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: Successful adult emergence (dose-effect relationship), mortality, qualitative observations: body size, remaining food.

Reference item: Dimethoate tech. (analyzed purity: 98.8% w/w).

Test doses: Control 1: untreated diet (50% aqueous yeast/sugar solution with 50% royal jelly containing 0.5% v/v water)

Control 2: untreated diet with acetone (0.5% v/v)

Test item treatments:

|  |  |
| --- | --- |
| **Nominal dose/concentration of BAS 700 F** | |
| **Doses [µg a.s./larva]** | **Concentrations [mg a.s./kg food]** |
| 5.0 | 32 |
| 10 | 63 |
| 20 | 126 |
| 40 | 253 |
| 80 | 506 |

Reference item: treated diet with a dose of 7.6 μg dimethoate/larva (corresponding concentration: 48 mg a.s./kg food)

Test conditions: Temperature (D1-D22):

34.0°C – 34.8°C

Relative humidity:

99 - 100% (D1-D8)

82 - 86% (D8 – D15)

60 - 68% (D15 – D22)

Photoperiod: darkness (except during assessments)

Food: 50% aqueous yeast/sugar solution with 50% royal jelly.

Analytics: Analytical verification of test item concentrations in honey bee larvae diet was conducted using an HPLC-method with MS-MS detection (Method No. L0383/01).

Statistics: Descriptive statistics; The Chi² Table Test with Bonferroni Correction (one-sided greater, α = 0.05) for determination of NOED/NOEC (D8 and D22).

**C. Description of the analytical procedures**

Concentrations of BAS 700 F in honey bee larvae diet were determined using the method described according to an in-house developed method L0383/01. For the extraction procedure 5 mL of water and 5 mL of acetonitrile as well as QuEChERS extraction mix, containing 0.5 g magnesium sulfate, 0.12 g sodium chloride, were added to a sample aliquot of 0.2 g. The mixture was shaken vigorously for 3 min with a Multitube-Vortexer and centrifuged for 2 minutes at 3000 g. Aliquots of the acetonitrile-phase were diluted and analyzed by HPLC-MS-MS. The limit of quantification (LOQ) was 14.3 mg/kg and the limit of detection (LOD) was set to 4.56 mg/kg. Matrix effects were taken into account by spiking the calibration solutions with 19% of QuEChERS blank extract obtained from extraction of 0.2 g of untreated sample matrix. Thus, all measuring samples contained the same amount of original sample matrix. Details on measured fortification samples and obtained procedural recoveries for BAS 700 F are given in the tables below.

Table A 21: Procedural recoveries for BAS 700 F (Mass Transition 382🡪 362)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level (mg/kg)** | **n** | **Mean recovery (%)** | **RSD (%)** |
| Honey bee larvae diet | 14.3 | 5 | 103 | 7.1 |
| Honey bee larvae diet | 682 | 5 | 88 | 9.8 |

**Table A 22: Procedural recoveries for BAS 700 F (Mass Transition 382🡪 342)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Fortification level (mg/kg)** | **n** | **Mean recovery (%)** | **RSD (%)** |
| Honey bee larvae diet | 14.3 | 5 | 103 | 7.6 |
| Honey bee larvae diet | 682 | 5 | 88 | 9.9 |

**II. RESULTS AND DISCUSSION**

After 120 hours of repeated oral exposure (on D8) larval mortalities of 0.0 and 2.8% were observed in the controls, respectively. Pupal mortality (between D8 and D22) was 19.4% in the untreated control and 17.1% in the solvent control. The control groups showed a total mortality of 19.4% in the untreated and in the solvent control, respectively, at D22. In the test item group, larval mortalities at D8 ranged between 0.0 and 2.8%. Pupal mortalities ranged between 14.3 and 41.7% in the test item treatment groups. Total mortalities at D22 ranged between 16.7 and 41.7%. On D8, no statistically significantly increased mortality occurred in any of the larvae groups after being treated with BAS 700 F (Chi² Table Test with Bonferroni Correction, one-sided greater, α = 0.05) and none individual of all remaining larvae showed remaining food.

In the final assessment at D22, adult emergence rates of 80.6% were determined for the honey bees in both control groups. In the test item group, the adult honey bees emerged at rates ranging between 58.3 and 83.3% following an application of 5.0, 10, 20, 40 and 80 μg a.s./larva, respectively, during the larval stages. No statistically significantly increased mortality occurred in any of the larvae groups after being treated with BAS 700 F (Chi² Table Test with Bonferroni Correction, one-sided greater, α = 0.05). The results are summarized in Table A 23.

Table A 23: Toxicity of BAS 700 F to *Apis mellifera* (honey bee) in a repeated exposure larval

toxicity test after 22 days

| **Dosage [µg a.s./larva]** | **Concentration [mg a.s./kg food]** | **D8 mortality** | | **D22 pupal mortality [%]** | | **D22 overall mortality [%]** | | **D22 adult emergence [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **abs.** | **corr.** 1) | **abs.** | **corr.** 1) | **abs.** | **corr.** 1) |
| Control | Control | 0.0 | -- | 19.4 | 0.0 | 19.4 | 0.0 | 80.6 |
| Acetone solvent control | Acetone solvent control | 2.8 | 0.0 | 17.1 | 0.0 | 19.4 | 0.0 | 80.6 |
| 5.0 | 32 | 2.8 | 0.0 | 14.3 | 0.0 | 16.7 | 0.0 | 83.3 |
| 10 | 63 | 0.0 | 0.0 | 19.4 | 2.8 | 19.4 | 0.0 | 80.6 |
| 20 | 126 | 0.0 | 0.0 | 16.7 | 0.0 | 16.7 | 0.0 | 83.3 |
| 40 | 253 | 2.8 | 0.0 | 28.6 | 13.8 | 30.6 | 13.8 | 69.4 |
| 80 | 506 | 0.0 | 0.0 | 41.7 | 29.6 | 41.7 | 27.6 | 58.3 |
| **Endpoints [D8]** | | | | | | | | |
| LD50 [µg a.s./larva] 2) | | >80 | | | | | | |
| NOEDmortality [µg a.s./larva] 3) | | ≥80 | | | | | | |
| LC50 [mg a.s./kg food] 2) | | >506 | | | | | | |
| NOECmortality [mg a.s./kg food] 3) | | ≥506 | | | | | | |
| **Endpoints [D22]** | | | | | | | | |
| ED50 [µg a.s./larva] 2) | | >80 | | | | | | |
| NOEDemergence [µg a.s./larva] 3) | | ≥80 | | | | | | |
| EC50 [mg a.s./kg food] 2) | | >506 | | | | | | |
| NOECemergence [mg a.s./kg food] 3) | | ≥506 | | | | | | |

Negative values are set to 0; abs.: absolute; corr.: corrected

1) Corrected mortality according to Schneider-Orelli (1947).

2) Estimated.

3) Chi² Table Test with Bonferroni Correction, one-sided greater, α = 0.05.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 239 (2016)** | **Obtained in this study** |
| Control mortality from D3 to D8 ≤ 15% across all replicates | 0% untreated control  2.8% solvent control |
| Adult emergence in the control group ≥ 70% at D22 across all replicates | 80.6% untreated control 80.6% solvent control |
| Effects of the reference item:  Dimethoate: larval mortality ≥ 50% on D8 across all replicates | 75.0% at D8 |

All validity criteria were met.

**III. CONCLUSION**

**In a repeated exposure larval toxicity study with BAS 700 F, the LD50 (larval mortality on D8) was estimated to be > 80 μg a.s./larva, which is equivalent to a LC50 of > 506 mg a.s./kg food. The respective NOED was ≥ 80 μg a.s./larva and the corresponding NOEC was ≥ 506 mg a.s./kg food. The ED50 (successful adult emergence up to D22) was estimated to be > 80 μg a.s./larva, which is equivalent to an EC50 of > 506 mg a.s./kg food. The respective NOED was ≥ 80 μg a.s./larva and the corresponding NOEC was ≥ 506 mg a.s./kg food.**

* + - * 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.1.3/2 |
| Report | Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 736 00 F under laboratory conditions,  Haeuser, R., 2021  report No 900787, S20-05930  2020/2081000  Authority registration No |
| Guideline(s): | OECD 239 (2016) |
| Deviations: | No |
| GLP: | yes  (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of the test item BAS 736 00 F on survival and adult emergence of honey bee larvae (*Apis mellifera*)were investigated in a laboratory test with repeated exposure over a time period of 22 days. Synchronized 1st larval stage (L1) honey bee larvae (*Apis mellifera*) were fed with artificial diet for 5 days (day 1, 3, 4, 5 and 6). On days 3, 4, 5 and 6, larvae were fed with diet containing five different concentrations of BAS 736 00 F resulting in concentrations of 520, 779, 1169, 1753 and 2630 mg product/kg food, corresponding to total doses of 80.1, 120, 180, 270 and 405 µg product/larva per developmental period. Untreated diet served as a control and dimethoate at a dose rate of 7.39 µg/larva served as reference item treatment. All treatment groups and control contained larvae from three different bee colonies. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of remaining food after 120 hours (D8) were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

After 120 hours of repeated oral exposure (on D8) no mortality was observed in the control. Pupal mortality (between D8 and D22) was 10.4% in the untreated control. The control groups showed a total mortality of 10.4% in the untreated control, at D22. In the test item group, larval mortalities at D8 ranged between 0.0 and 85.4%. Pupal mortalities ranged between 10.4 and 100% in the test item treatment groups. Total mortalities at D15 ranged between 6.3 and 100%. A statistically significant difference was found at test item concentrations of 1169, 1753 and 2630 mg product/kg diet (corresponding to cumulative nominal doses of 180, 270 and 405 µg product/larva per developmental period) compared to the control. Compared to the control group, emerged bees of the test item group exposed to 1169 mg product/kg foot had a reduced body size on day 22. On D8 uneaten food was observed in the control group, all test item groups and the reference item group. In the final assessment at D22, adult emergence rates of 89.6% were determined for the honey bees in the control group. In the test item group, the adult honey bees emerged at rates ranging between 54.2 and 89.6% following an application of 80.1, 120, 180, 270 and 405 µg product/larva per developmental period. A statistically significant difference was found at test item concentrations of 1169, 1753 and 2630 mg product/kg diet (corresponding to cumulative nominal doses of 180, 270 and 405 µg product/larva per developmental period) compared to the control.

**In a repeated exposure larval toxicity study with BAS 736 00 F, the LD50 (larval mortality on D8) was estimated to be 313 μg product/larva, which is equivalent to a LC50 of 2031 mg product/kg food. The respective NOED was 120 μg product/larva and the corresponding NOEC was 779 mg product/kg food. The ED50 (successful adult emergence up to D22) was estimated to be 188 μg product/larva, which is equivalent to an EC50 of 1222 mg product/kg food. The respective NOED was 120 μg product/larva and the corresponding NOEC was 779 mg product/kg food.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Larvae of honey bee(*Apis mellifera* L.); synchronized first larval stage instar (L1); derived from at least three healthy and queen-right colonies adequately fed, healthy, as far as possible parasite-free; source: test facility own stock.

Test design: 22-day repeated exposure larval toxicity test according to OECD 239 (2016). L1 honey bee larvae (*Apis mellifera*)were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment (on D1). After this, the larvae were fed during larval development with artificial diet, containing the test item on rearing days 3, 4, 5 and 6. In total, 5 treatment groups were set up: 5 doses of the test item, 1 untreated control group, 1 and 1 dose of the reference item; each with 3 replicates and 16 larvae per replicate. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of remaining food after 120 hours (D8) were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: Successful adult emergence (dose-effect relationship, EC/D50, NOEC/D), Mortality (LC/D50), qualitative observations: body size, remaining food.

Reference item: BAS 152 I (dimethoate, analyzed purity: 99.0% w/w).

Test doses: Control: untreated diet (50% aqueous yeast/sugar solution with 50% royal jelly)

Test item treatments:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Concentration [mg/kg food] | | | | Dose [µg/larva/developmental period] | | |
| BAS 736 00 F | BAS 9164 F | BAS 700 F | BAS 736 00 F | | BAS 9164 F | BAS 700 F |
| 520 | 36.2 | 24.1 | 80.1 | | 5.57 | 3.71 |
| 779 | 54.2 | 36.1 | 120 | | 8.35 | 5.56 |
| 1169 | 81.4 | 54.2 | 180 | | 12.5 | 8.35 |
| 1753 | 122 | 81.3 | 270 | | 18.8 | 12.5 |
| 2630 | 183 | 122 | 405 | | 28.2 | 18.8 |

Reference item: treated diet with a dose of 7.39 μg a.s./larva per developmental period (corresponding concentration: 48 mg a.s./kg food).

Test conditions: Temperature:

Day 1 to day 8: Mean of 34.2 °C (T1 to T5 and C) and 34.4 °C (R)

Day 8 to day 15: Mean of 35.1 °C (T1 to T5 and C)

Day 15 to day 22: Mean of 35.5 °C (T1 to T5 and C)\*

(Target: 34 - 35 °C, but not below 23 °C or above 40 °C)

Relative humidity:

Day 1 to day 8: Means of 99.1% (T1 to T5 and R) and 99.4% (C)

Day 8 to day 15: Mean of 85.6%\* for C and T1 to T5

Day 15 to day 22: Mean of 70.0% for C and T1 to T5

\*with deviations ≥ 2 h but having no impact on study outcome

Photoperiod: darkness (except during grafting, feeding and assessments)

Food: 50% aqueous yeast/sugar solution with 50% royal jelly.

Analytics: Analytical verification of the test item concentrations in honey bee larvae diet was conducted by BASF method L0372/02 using liquid chromatography with mass-spectrometric (LC-MS/MS) detection.

Statistics: Descriptive statistics; Cochran-Armitage test with Rao-Scott adjustment (one-sided greater, α = 0.05) and Weibull analysis for mortality; Cochran-Armitage test (one-sided greater, α = 0.05) and Weibull analysis (compensated for control response) for adult emergence determination.

**C. DESCRIPTION OF THE ANALYTICAL PROCEDURES**

Concentration control analysis of BAS 700 F as representative active ingredient of BAS 736 00 F in honey bee larval diet was determined according to the analytical method L0372/02. The validation of the analytical method is described in the study report. About 2.2 g of thawed and vortexed samples were extracted with 44 mL methanol/water (3:1, v/v). After 30 s of shaking the mixture and 10 min of centrifuging at 2500 rpm, the extract was decanted into a 250 mL graduated cylinder and filled up to the 110 mL mark with methanol/water (3:1, v/v). After shaking it by hand, 1 mL of the sample extract was then transferred into a PSA-Kit-3a and it was vortexed for another 30 s. The samples were centrifuged at maximum rpm for 5 min and then diluted with methanol/water (3:1, v/v). If necessary, the diluted extracts were further diluted into the range of the calibration curve before injecting into the HPLC system. The determination was performed by LC-MS/MS. The limit of quantification (LOQ) was 0.0102 mg/kg and the limit of detection (LOD) was 0.0025 mg/kg. Matrix effects were compensated by using matrix-matched calibration standards with solvent standards (= standards in methanol/water (3:1, v/v)) at identical nominal concentrations. The effects were assessed by comparing the standard’s peak areas. Since the samples of the test item feeding solutions were analyzed within 49 days, the analysis of storage stability samples did not take place. Details on measured fortification samples and obtained procedural recoveries for BAS 736 00 F are given in Table A 24.

**Table A 24: Procedural recoveries for BAS 00 F in honey bee food**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Substance** | **Matrix** | **Test item Fortification level (mg/kg)** | **Fortification level Fluxapyroxad (mg/kg)** | **n** | **Mean (%)** | **RSD (%)** |
| BAS 00 F | Honey bee larvae food | 0.219 | 0.0102 | 7 | 91.8 | 13 |
| 3500 | 162 | 5 | 105 | 7.5 |

*RSD = relative standard deviation*

**II. RESULTS AND DISCUSSION**

After 120 hours of repeated oral exposure (on D8) no mortality was observed in the control. Pupal mortality (between D8 and D22) was 10.4% in the untreated control. The control groups showed a total mortality of 10.4% in the untreated control, at D15. In the test item group, larval mortalities at D8 ranged between 0.0 and 85.4%. Pupal mortalities ranged between 10.4 and 100% in the test item treatment groups. Total mortalities at D15 ranged between 6.3 and 100%. A statistically significant difference was found at test item concentrations of 1169, 1753 and 2630 mg product/kg diet (corresponding to cumulative nominal doses of 180, 270 and 405 µg product/larva per developmental period) compared to the control (Cochran-Armitage test with Rao-Scott adjustment: one-sided greater, α = 0.05). Compared to the control group, emerged bees of the test item group exposed to 1169 mg product/kg foot had a reduced body size on day 22. On D8 uneaten food was observed in the control group, all test item groups and the reference item group.

In the final assessment at D22, adult emergence rates of 89.6% were determined for the honey bees in the control group. In the test item group, the adult honey bees emerged at rates ranging between 0.0 and 89.6% following an application of 80.1, 120, 180, 270 and 405 µg product/larva per developmental period. A statistically significant difference was found at test item concentrations of 1169, 1753 and 2630 mg product/kg diet (corresponding to cumulative nominal doses of 180, 270 and 405 µg product/larva per developmental period) compared to the control (Cochran-Armitage test: one-sided greater, α = 0.05). The results are summarized in Table A 25.

Table A 25: Toxicity of BAS 736 00 F to *Apis mellifera* (honey bee) in a repeated exposure larval

toxicity test after 22 days

| **Dosage [µg product/larva]** | **Concentration [mg product/kg food]** | **D8 mortality**  **[%]** | | **D22 pupal mortality [%]** | | **D15 overall mortality [%]** | | **D22 adult emergence [%]** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **abs.** | **corr.** 1) | **abs.** | **corr.** 1) | **abs.** | **corr.** 1) |
| Control | Control | 0.0 | -- | 10.4 | -- | 10.4 | -- | 89.6 |
| 80.1 | 520 | 0.0 | 0.0 | 10.4 | 0.0 | 6.3 | -4.6 | 89.6 |
| 120 | 779 | 0.0 | 0.0 | 14.6 | 4.7 | 10.4 | 0.0 | 85.4 |
| 180 | 1169 | 12.5\* | 12.5\* | 38.1 | 30.9 | 33.3 | 25.6 | 54.2\*\* |
| 270 | 1753 | 29.2\* | 29.2\* | 100.0 | 100.0 | 70.8 | 67.4 | 0.0\*\* |
| 405 | 2630 | 85.4\* | 85.4\* | 100.0 | 100.0 | 100.0 | 100.0 | 0.0\*\* |
| **Endpoints [D8]** | | | | | | | | |
| LD50 [µg product/larva per developmental period] 2) | | 313  (95% lower - upper CL = 290 - 336) | | | | | | |
| NOEDmortality [µg product/larva per developmental period] 2) | | 120 | | | | | | |
| LC50 [mg product/kg food] | | 2031  (95% lower - upper CL = 1880 - 2180) | | | | | | |
| NOECmortality [mg product/kg food] | | 779 | | | | | | |
| **Endpoints [D22]** | | | | | | | | |
| ED50 [µg product/larva per developmental period] 2) | | 188  (95% lower - upper CL = 177 - 204) | | | | | | |
| NOEDmortality [µg product/larva per developmental period] 2) | | 120 | | | | | | |
| EC50 [mg product/kg food] | | 1222  (95% lower - upper CL = 1147 - 1322) | | | | | | |
| NOECmortality [mg product/kg food] | | 779 | | | | | | |

Negative values are set to 0; abs.: absolute; corr.: corrected; CL: confidence limit

\* Statistically significantly different compared to control group (Cochran-Armitage test with Rao-Scott adjustment: one sided greater, α = 0.05).

\*\* Statistically significantly different compared to control group (Cochran-Armitage test: one sided greater, α = 0.05).

1) Corrected cumulative mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

2) Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm3.

On D8, the mortality in the reference item treatment was 87.5%.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 239 (2016)** | **Obtained in this study** |
| Control mortality from D3 to D8 ≤ 15% across all replicates | 0% control |
| Adult emergence in the control group ≥ 70% at D22 across all replicates | 89.6% control |
| Effects of the reference item:  Dimethoate: larval mortality ≥ 50% on D8 across all replicates | 87.5% at D8 |

All validity criteria were met.

**III. CONCLUSION**

**In a repeated exposure larval toxicity study with BAS 736 00 F, the LD50 (larval mortality on D8) was estimated to be 313 μg product/larva, which is equivalent to a LC50 of 2031 mg product/kg food. The respective NOED was 120 μg product/larva and the corresponding NOEC was 779 mg product/kg food. The ED50 (successful adult emergence up to D22) was estimated to be 188 μg product/larva, which is equivalent to an EC50 of 1222 mg product/kg food. The respective NOED was 120 μg product/larva and the corresponding NOEC was 779 mg product/kg food.**

* + - 1. KCP 10.3.1.4 Sub-lethal effects

As BAS 736 00 F does not pose an unacceptable risk to honey bees, further studies are not necessary.

* + - 1. KCP 10.3.1.5 Cage and tunnel tests

As BAS 736 00 F does not pose an unacceptable risk to honey bees, further studies are not necessary.

* + - 1. KCP 10.3.1.6 Field tests with honeybees

As BAS 736 00 F does not pose an unacceptable risk to honey bees, further studies are not necessary.

* + 1. KCP 10.3.2 Effects on non-target arthropods other than bees
    2. 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.2.1/1 |
| Report | Effects of BAS 736 00 F on the predatory mite Typhlodromus pyri SCHEUTEN in a laboratory test,  Roehlig, U., 2019  report No 849668, 1948NTL0009  2019/1061102  Authority registration No |
| Guideline(s): | IOBC (Bluemel et al. 2000) |
| Deviations: | No |
| GLP: | no  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a worst-case laboratory study, protonymphs of *Typhlodromus pyri* Scheuten (Acaria: Phytoseiidae) were exposed to dried residues of BAS 736 00 F on glass plates. The test item was applied at test rates of 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha. Additional test units were treated with deionized water as control and with Dimethoate EC 400 (dimethoate) as reference item. Mortality was assessed 3 and 7 days after treatment by the number of surviving, dead and escaped predatory mites.

After 7 days, in the water-treated control a mortality of 2.0% was observed. In the test item treatments mortality ranged between 1.0% and 100.0%. This resulted in corrected mortality rates between -1.0% and 100%. Statistically significant differences in mortality compared to the control were observed at 2.0 and 4.0 L BAS 736 00 F/ha.

**In a worst-case laboratory study with BAS 736 00 F, the LR50 for *Typhlodromus pyri* was determined to be 2.75 L BAS 736 00 F/ha.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Predatory mite (*Typhlodromus pyri* Scheuten), protonymphs, less than 24 hours old, source: source (in the stage of eggs): Katz Biotech AG, 15837 Baruth, Germany.

Test design: Exposure of protonymphs via air-dried residues on treated glass plates; 7 treatment groups (5 test item rates, 1 water treated control and 1 reference item) with 5 replicates (consisting of 20 protonymphs) per treatment; Assessments of mortality on day 3 and 7 after treatment.

Endpoints: Mortality after exposure over 7 days, including determination of the LR50 (Lethal Rate 50%, rate resulting in 50% mortality).

Reference item: Dimethoate EC 400 (a.s.: dimethoate, analyzed content: 429.0 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha, reference item: 15 mL/ha.

|  |  |  |
| --- | --- | --- |
| BAS 736 00 F  [L product/ha] | BAS 9164 F  [g a.s./ha]\* | BAS 700 F  [g a.s./ha]\* |
| 0.25 | 18.75 | 12.5 |
| 0.5 | 37.5 | 25 |
| 1.0 | 75 | 50 |
| 2.0 | 150 | 100 |
| 4.0 | 300 | 200 |

\* based on nominal content of active substance and a test item density of 1.078 g/cm3.

All substances were applied in 200 L water/ha and sprayed onto glass plates via a laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 23 °C – 27 °C, relative humidity: 67% - 73%; photoperiod: 16 h light : 8 h dark; light intensity: 2070 lux; food: pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics, Chi2 2x2 Table Test with Bonferroni Correction (α = 0.05) for mortality, Weibull analysis for LR50 calculation.

**II. RESULTS AND DISCUSSION**

After 7 days, in the water-treated control a mortality of 2.0% was observed. In the test item treatments mortality ranged between 1.0% and 100.0%. This resulted in corrected mortality rates between -1.0% and 100%. Statistically significant differences in mortality compared to the control were observed at 2.0 and 4.0 L BAS 736 00 F/ha (Chi2 2x2 Table Test with Bonferroni Correction, α = 0.05). The results are summarized below in Table A 26.

Table A 26: Effects on predatory mites (*Typhlodromus pyri*) exposed to BAS 736 00 F in a

laboratory trial

|  |  |  |  |
| --- | --- | --- | --- |
| **Test item** | **Rate1)  [L/ha]** | **Mortality2) [%]** | **Corrected mortality3)  [%]** |
| Control | -- | 2.0 | -- |
| BAS 736 00 F | 0.25 | 1.0 | -1.0 |
| 0.5 | 2.0 | 0 |
| 1.0 | 3.0 | 1.0 |
| 2.0 | 10.0 \* | 8.2 |
| 4.0 | 100 \* | 100 |
|  | **Endpoint [L BAS 736 00 F/ha]** | | |
| LR50  [95% CL] | 2.75  [2.53-3.01] | | |

*CL = Confidence Limits*

\* Mortality statistically significant different compared to the control (Chi2 2x2 Table Test with Bonferroni Correction, α = 0.05).

1) Application rate in 200 L water/ha.

2) Mortality after 7 days of exposure to BAS 736 00 F on glass surface.

3) Corrected mortality according to Abbott (1925).

The reference item caused a mortality of 78.0% of exposed mites, resulting in a corrected mortality of

77.6%.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to Bluemel et al (2000)** | **Obtained in this study** |
| Control mortality ≤ 20% on day 7 | 2.0% |
| Corrected mortality in the reference group 50-100% on day 7 | 77.6% |

All validity criteria were met.

**III. CONCLUSION**

**In a worst-case laboratory study with BAS 736 00 F, the LR50 for *Typhlodromus pyri* was determined to be 2.75 L BAS 736 00 F/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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.2.1/2 |
| Report | Effects of BAS 736 00 F on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in a laboratory test,  Roehlig, U., 2019  report No 849671, 1948NAL0009  2019/1061100  Authority registration No |
| Guideline(s): | Mead-Briggs M. et al. (2000) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a worst-case laboratory study, adultsof *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to dried residues of 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha. Additional test units were treated with deionized water as control and with Dimethoate EC 400 (dimethoate) as reference item. Mortality was assessed 2, 24 and 48 hours after exposure.

After 48 hours in the water-treated control a mortality of 5.0% was observed. In the test item treatments mortality ranged between 5.0% and 92.5%. This resulted in corrected mortality rates between 0% and 92.1%. Statistically significant differences in mortality compared to the control were observed at 2.0 and 4.0 L BAS 736 00 F/ha.

**In a worst-case laboratory study with BAS 736 00 F, the LR50 for *Aphidius rhopalosiphi* was determined to be 2.67 L BAS 736 00 F/ha.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Parasitic wasp (*Aphidius rhopalosiphi* DeStephani-Perez), adults (< 48 hours old); source (in the stage of mummies): Katz Biotech AG, 15837 Baruth, Germany.

Test design: Exposure of parasitoids via air-dried residues on treated glass plates; 7 treatment groups (5 test item rates, water treated control, reference item) with 4 replicates per treatment; each replicate containing 7 females and 3 males. Assessment of mortality 2, 24 and 48 hours after test initiation.

Endpoints: Mortality after exposure over 48 hours including the determination of LR50.

Reference item: Dimethoate EC 400 (a.s.: dimethoate, analyzed content: 429.0 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha, reference item: 0.3 mL/ha.

|  |  |  |
| --- | --- | --- |
| BAS 736 00 F  [L product/ha] | BAS 9164 F  [g a.s./ha]\* | BAS 700 F  [g a.s./ha]\* |
| 0.25 | 18.75 | 12.5 |
| 0.5 | 37.5 | 25 |
| 1.0 | 75 | 50 |
| 2.0 | 150 | 100 |
| 4.0 | 300 | 200 |

\* based on nominal content of active substance and a test item density of 1.078 g/cm3.

All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates via laboratory spraying equipment and left air dried afterwards.

Test conditions: Temperature: 19.0 °C - 22.0 °C; relative humidity: 68% - 73%; photoperiod: 16 h light : 8 h dark; light intensity: 2010 lux: food: 25% w/w aqueous fructose solution.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics. Step-down Cochran-Armitage Test (α = 0.05) for mortality, Weibull analysis for LR50 calculation.

**II. RESULTS AND DISCUSSION**

After 48 hours in the water-treated control a mortality of 5.0% was observed. In the test item treatments mortality ranged between 5.0% and 92.5%. This resulted in corrected mortality rates between 0% and 92.1%. Statistically significant differences in mortality compared to the control were observed at 2.0 and 4.0 L BAS 736 00 F/ha (Step-down Cochran-Armitage Test, α = 0.05). The results are summarized in Table A 27.

Table A 27: Effects on parasitoids (*Aphidius rhopalosiphi*) exposed to BAS 736 00 F in a

laboratory trial

|  |  |  |  |
| --- | --- | --- | --- |
| **Test item** | **Rate1)  [L/ha]** | **Mortality2) [%]** | **Corrected mortality3)  [%]** |
| Control | -- | 5.0 | -- |
| BAS 736 00 F | 0.25 | 5.0 | 0 |
| 0.5 | 5.0 | 0 |
| 1.0 | 12.5 | 7.9 |
| 2.0 | 22.5 \* | 18.4 |
| 4.0 | 92.5 \* | 92.1 |
|  | **Endpoint [L BAS 736 00 F/ha]** | | |
| LR50  [95% CL] | 2.67  [2.34-2.98] | | |

*CL = Confidence Limits*

\* Mortality statistically significant different compared to the control (Step-down Cochran-Armitage Test, α = 0.05).

1) Application rate in 200 L water/ha.

2) Mortality after 48 hours of exposure to BAS 736 00 F on glass surface.

3) Corrected mortality according to Abbott (1925).

The reference item caused a mortality of 100% of exposed wasps, resulting in a corrected mortality of

100%.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to Mead-Briggs M. et al. (2000)** | **Obtained in this study** |
| Control mortality < 13% (48h) | 5.0% |
| Corrected mortality in the reference item group 50 - 100% (48h) | 100% |

All validity criteria were met.

**III. CONCLUSION**

**In a worst-case laboratory study with BAS 736 00 F, the LR50 for *Aphidius rhopalosiphi* was determined to be 2.67 L BAS 736 00 F/ha.**

* + 1. KCP 10.3.2.2 Extended laboratory testing, aged residue studies with 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.2.2/1 |
| Report | Effects of BAS 736 00 F on the predatory mite Typhlodromus pyri Scheuten in an extended laboratory test,  Roehlig, U., 2019  report No 849669, 1948NTE0009  2019/1061104  Authority registration No |
| Guideline(s): | IOBC (Bluemel et al. 2000) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In an extended laboratory study, protonymphs of *Typhlodromus pyri* Scheuten (Acarina: Phytoseiidae) were exposed to fresh, dried residues of BAS 736 00 F on bean leaf discs. The test item was applied at application rates of 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha. Additional test units were treated with deionized water as control and with Dimethoate EC 400 (dimethoate) as reference item. Mortality was assessed on after 7 days. Reproduction was assessed between 7 - 14 days by the number of eggs laid and number of juveniles per evaluation period

After 7 days of exposure, 2.0% mortality was observed in the control group. In the test item treatments mortality ranged between 1.0% and 3.0%. This resulted in corrected mortality rates between -1.0% and 1.0%. No statistically significant effects on mortality were determined at rates up to and including 4.0 L/ha BAS 736 00 F compared to the control. The reproduction rate amounted to 6.63 eggs/female in the control treatment. The reproduction rate in the different test item treated groups ranged from 6.11 to 6.88 eggs/female. Thus, an effect on reproduction between -3.8% and 7.8% was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at rates up to and including 4.0 L/ha BAS 736 00 F.

**In an extended laboratory study with BAS 736 00 F, the LR50 for *Typhlodromus pyri* was > 4 L BAS 736 00 F/ha. The ER50** **for reproduction was estimated to be > 4 L BAS 736 00 F/ha.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Predatory mite (*Typhlodromus pyri* Scheuten), protonymphs, (< 24 hours old); source (in the stage of eggs): Katz Biotech AG, 15837 Baruth, Germany.

Test design: Exposure of the predatory mites was reached via air-dried residues on on bean leaf discs (*Phaseolus vulgaris*). The following 7 treatment groups were set up: 5 test item rates, water treated control, reference item with 5 replicates (consisting of 20 protonymphs) per treatment. Assessment of mortality on day 3 and 7. For the control and all rates up to and including 4.0 L/ha BAS 736 00 F, the reproduction, i.e. number of eggs per female, was determined (3 assessments, 9, 11 and 14 days after application).

Endpoints: LR50: lethal rate producing 50% mortality after exposure over 7 days, ER50: effect rate resulting in 50% effect on reproduction between 7 - 14 days.

Reference item: Dimethoate EC 400 (a.s.: dimethoate, analyzed content: 429.0 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha, reference item: 30 mL/ha.

|  |  |  |
| --- | --- | --- |
| BAS 736 00 F  [L product/ha] | BAS 9164 F  [g a.s./ha]\* | BAS 700 F  [g a.s./ha]\* |
| 0.25 | 18.75 | 12.5 |
| 0.5 | 37.5 | 25 |
| 1.0 | 75 | 50 |
| 2.0 | 150 | 100 |
| 4.0 | 300 | 200 |

\* based on nominal content of active substance and a test item density of 1.078 g/cm3.

All substances were applied in 200 L water/ha and sprayed on the bean via a laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 23 °C – 27 °C; relative humidity: 68% – 73%; photoperiod: 16 h light : 8 h dark; light intensity: 1950 lux; food: pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics, Chi2 2x2 Table test with Bonferroni Correction (α = 0.05) for mortality, Williams t-test for reproductive capacity (α = 0.05).

**II. RESULTS AND DISCUSSION**

After 7 days of exposure, 2.0% mortality was observed in the control group. In the test item treatments mortality ranged between 1.0% and 3.0%. This resulted in corrected mortality rates between -1.0% and 1.0%. No statistically significant effects on mortality were determined at rates up to and including 4.0 L/ha BAS 736 00 F compared to the control (Chi2 2x2 Table test with Bonferroni Correction, α= 0.05). The reproduction rate amounted to 6.63 eggs/female in the control treatment. The reproduction rate in the different test item treated groups ranged from 6.11 to 6.88 eggs/female. Thus, an effect on reproduction between -3.8% and 7.8% was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at rates up to and including 4.0 L/ha BAS 736 00 F (Williams t-test, α = 0.05).

The results are summarized in Table A 28.

Table A 28: Effects on predatory mites (*Typhlodromus pyri*) exposed to BAS 736 00 F in an

extended laboratory trial

| **Treatment** | **Rate  [L/ha] 1)** | **Mortality  [%] 2)** | **Mortality corr.  [%] 3)** | **Reproduction [eggs/female] 4)** | **Effects on reproduction [%] 5)** |
| --- | --- | --- | --- | --- | --- |
| Control | -- | 2.0 | -- | 6.63 | -- |
| BAS 736 00 F | 0.25 | 2.0 | 0 | 6.34 | 4.4 |
| 0.5 | 2.0 | 0 | 6.57 | 0.9 |
| 1.0 | 1.0 | -1.0 | 6.39 | 3.6 |
| 2.0 | 1.0 | -1.0 | 6.88 | -3.8 |
| 4.0 | 3.0 | 1.0 | 6.11 | 7.8 |
|  | **Endpoints [L BAS 736 00 F/ha]** | | | | |
| LR50 | > 4.0 | | | | |
| ER50 | > 4.0 | | | | |

1) Application rate in 200 L water/ha.

2) Mortality after 7 days of exposure to BAS 736 00 F on treated bean leaves according to Chi2 2x2 Table test with Bonferroni Correction (α = 0.05).

3) Corrected mortality according to Abbott (1925).

4) Reproduction: mean number of eggs/female from day 7 to 14 (Williams t-test, α = 0.05).

5) Change in numbers of eggs per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase, relative to the control.

The reference item caused a mortality of 74.0% of exposed mites, resulting in a corrected mortality of

73.5%.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to Bluemel et al (2000)** | **Obtained in this study** |
| Control mortality ≤ 20% on day 7 | 2.0% |
| Corrected mortality in the reference group 50-100% on day 7 | 73.5% |
| Number of eggs in the control ≥ 4 eggs/female | 6.63 |

All validity criteria were met.

**III. CONCLUSION**

**In an extended laboratory study with BAS 736 00 F, the LR50 for *Typhlodromus pyri* was > 4 L BAS 736 00 F/ha. The ER50 for reproduction was estimated to be > 4 L BAS 736 00 F/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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.2.2/2 |
| Report | Effects of BAS 736 00 F on the parasitic wasp Aphidius rhopalosiphi (Destefani-Perez) in an Extended laboratory test,  Roehlig, U., 2019  report No 849672, 1948NAE0010  2019/1061101  Authority registration No |
| Guideline(s): | IOBC (Mead-Briggs et al. 2009) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In an extended laboratory study, adults of the parasitic wasp *Aphidius rhopalosiphi* were exposed to fresh, dried residues of BAS 736 00 F on potted barley plants. The test item was applied at application rates of 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha. Additional test units were treated with deionized water as control and with Dimethoate EC 400 (dimethoate) as reference item. Assessment of mortality of wasps was conducted 2, 24 and 48 h after test initiation by the number of surviving, affected, moribund and dead wasps. For the reproduction assessment 15 females from each treatment were transferred individually to pots with untreated, aphid-infested wheat plants for 24 h and then removed. The number of parasitized aphid mummies was recorded 11 days later.

After 48 hours, in the water-treated control a mortality of 6.7% was observed. In the test item treatments mortality ranged between 3.3% and 10.0%. This resulted in corrected mortality rates between -3.6% and 3.6%. No statistically significant effects on mortality were determined in all test item treatments. The mean number of mummies per female in the test item treatments was between 20.1 and 23.3 in comparison to the control with 21.6 mummies per female. No statistically significant effects on reproductive capacity were determined in all test item treatments.

**In an extended laboratory study with BAS 736 00 F, the LR50 for *Aphidius rhopalosiphi* was > 4.0 L BAS 736 00 F/ha. The ER50 for reproduction was estimated to be > 4.0 L BAS 736 00 F/ha.**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Parasitic wasp (*Aphidius rhopalosiphi* DeStephani-Perez), adults (< 48 hours old); source (in the stage of mummies): Katz Biotech AG, 15837 Baruth, Germany.

Test design: Exposure of the adults was achieved via air-dried spray residues on treated, potted barley plants. 7 treatment groups (5 test item rates, water treated control, reference item) with 6 replicates (consisting of 5 females) per treatment. Mortality assessments were carried out 2, 24 and 48 hours after start of exposure of the wasps. At 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). Assessment of reproduction capacity, i.e. number of mummies per female, was made for the control and all treated groups (1 assessment, 14 days after application).

Endpoints: Mortality: number of dead wasps, including the determination of the LR50. Reproductive capacity: number of mummies per female, including the determination of the ER50.

Reference item: Dimethoate EC 400 (a.s.: dimethoate, analyzed content: 429.0 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha, reference item: 10 mL/ha.

|  |  |  |
| --- | --- | --- |
| BAS 736 00 F  [L product/ha] | BAS 9164 F  [g a.s./ha]\* | BAS 700 F  [g a.s./ha]\* |
| 0.25 | 18.75 | 12.5 |
| 0.5 | 37.5 | 25 |
| 1.0 | 75 | 50 |
| 2.0 | 150 | 100 |
| 4.0 | 300 | 200 |

\* based on nominal content of active substance and a test item density of 1.078 g/cm3.

All substances were applied in 400 L water/ha. The substances were sprayed on potted barley plants via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 19 °C - 22 °C; relative humidity 67% - 73%; photoperiod 16 h light : 8 h dark; light intensity: 1020 lux (mortality phase), 3010 lux (parasitization phase), 6940 lux (reproduction phase); food: 10% w/w aqueous fructose solution.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics, Multiple Sequentially-rejective Fisher test after Bonferroni-Holm (α = 0.05) for mortality, Williams t-test for reproductive capacity (α = 0.05).

**II. RESULTS AND DISCUSSION**

After 48 hours, in the water-treated control a mortality of 6.7% was observed. In the test item treatments mortality ranged between 3.3% and 10.0%. This resulted in corrected mortality rates between -3.6% and 3.6%. No statistically significant effects on mortality were determined in all test item treatments (Multiple Sequentially-rejective Fisher test after Bonferroni-Holm, α = 0.05). The mean number of mummies per female in the test item treatments was between 20.1 and 23.3 in comparison to the control with 21.6 mummies per female. No statistically significant effects on reproductive capacity were determined in all test item treatments (Williams t-test, α = 0.05).

The results are summarized in Table A 29.

Table A 29: Effects of BAS 736 00 F on parasitoids (*Aphidius rhopalosiphi*) in an extended

laboratory trial

| **Treatment** | **Rate [L/ha] 1)** | **Mortality [%] 2)** | **Mortality corr.  [%] 3)** | **Reproduction  [mummies/female] 4)** | **Effect on Reproduction [%] 5)** |
| --- | --- | --- | --- | --- | --- |
| Control | -- | 6.7 | -- | 21.6 | -- |
| BAS 736 00 F | 0.25 | 6.7 | 0.0 | 21.6 | 0 |
| 0.5 | 3.3 | -3.6 | 23.3 | -7.9 |
| 1.0 | 6.7 | 0 | 20.1 | 6.9 |
| 2.0 | 10.0 | 3.6 | 20.6 | 4.6 |
| 4.0 | 6.7 | 0 | 22.2 | -2.8 |
|  | **Endpoints [L BAS 736 00 F/ha]** | | | | |
| LR50 | > 4.0 | | | | |
| ER50 | > 4.0 | | | | |

1) Application rate in 400 L water/ha.

2) Mortality after 48 h of exposure to BAS 736 00 F on treated barley plants.

3) Corrected mortality according to Abbott (1925).

4) Reproduction: mean number of parasitized aphids (mummies)/surviving female. The result is compared to a control (Williams t-test, α = 0.05).

5) Change in mean number of mummies per female, relative to control. A negative value indicates an increase and a positive value indicates a decrease relative to the control.

The reference item caused a mortality of 96.7% of exposed wasps, resulting in a corrected mortality of 96.4%.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to Mead-Briggs M. et al. (2009)** | **Obtained in this study** |
| Control mortality < 10% (48h) | 6.7% |
| Corrected mortality in the reference item group 50-100% (48h) | 96.4% |
| Number of mummies in the control ≥ 5 mummies/female and no more than two zero values | 21.6; no more than 2 zero values |

All validity criteria were met.

**III. CONCLUSION**

**In an extended laboratory study with BAS 736 00 F, the LR50 for *Aphidius rhopalosiphi* was > 4.0 L BAS 736 00 F/ha. The ER50 for reproduction was estimated to be > 4.0 L BAS 736 00 F/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. |

|  |  |
| --- | --- |
| Reference: | CP 10.3.2.2/3 |
| Report | Effects of BAS 736 00 F on the green lacewing Chrysoperla carnea STEPH. in an (extended laboratory test),  Roehlig, U., 2020  report No 849666, 2048NCE0002  2019/2054735  Authority registration No |
| Guideline(s): | IOBC (Vogt et al. 2000) modified for the exposure on natural substrate (extended laboratory test) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In an extended laboratory study, larvaeof *Chrysoperla carnea* (Neuroptera: Chrysopidae) were exposed to fresh, dried residues of BAS 736 00 F on bean leaves. The test item was applied at rates of 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha. Additional test units were treated with deionized water as control and with DANADIM PROGRESS (dimethoate) as reference item. Assessment of mortality was conducted regularly until hatching of the adult lacewings by the number of dead larvae and pupae. For the control and all test item groups up to and including 4.0 L/ha BAS 736 00 F the reproductive performance, i.e. egg deposition and hatching rate, was assessed (2 assessments/week, 24 h period each).

A mortality of 6.0% was observed in the control group. In the test item treatment groups, mortalities ranged from 6.0 to 50.0% resulting in corrected mortality rates between 0% and 46.8%. Statistically significant effects on mortality were observed in the highest test item treatment group of 4.0 L product/ha. No effects on reproduction of *Chrysoperla carnea* occurred, when the test item was applied at rates up to and including 4.0 L/ha BAS 736 00 F. Reproduction in the control and all test item treatments number of eggs per female per day was > 15 and the hatching rate was > 70% in the control.

**In an extended laboratory study with BAS 736 00 F, the LR50 for *Chrysoperla carnea* was > 4 L BAS 736 00 F/ha. No unacceptable effects on reproduction were observed up to and including an application rate of 4 L BAS 736 00 F/ha.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Green lacewing (*Chrysoperla carnea* Steph), larvae (2 - 3 days old), source: reared in the laboratory of the test facility.

Test design: Exposure of the larvae was reached via air-dried spray residues on treated bean leaves(*Phaseolus vulgaris*). 7 treatment groups (5 test item, water treated control, reference item); 50 replicates (consisting of one larva per replicate) per treatment group. Exposure lasted until pupae were transferred to oviposition units for development of adults. Mortality assessments were carried out regularly until hatching of the adult lacewings. In addition, for the control and all test item groups up to and including 4.0 L/ha BAS 736 00 F the reproductive performance, i.e. egg deposition and hatching rate, was determined (2 assessments/week, 24 h period each).

Endpoints: Pre-imaginal mortality including the estimation of a LR50 (Lethal Rate 50%, rate resulting in 50% mortality), Reproductive performance: number of produced eggs per female per day and hatching rate.

Reference item: DANADIM PROGRESS (a.s.: dimethoate, analyzed content: 411.2 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.25, 0.5, 1.0, 2.0 and 4.0 L BAS 736 00 F/ha, reference item: 40 mL/ha.

|  |  |  |
| --- | --- | --- |
| BAS 736 00 F  [L product/ha] | BAS 9164 F  [g a.s./ha]\* | BAS 700 F  [g a.s./ha]\* |
| 0.25 | 18.75 | 12.5 |
| 0.5 | 37.5 | 25 |
| 1.0 | 75 | 50 |
| 2.0 | 150 | 100 |
| 4.0 | 300 | 200 |

\* based on nominal content of active substance and a test item density of 1.078 g/cm3.

All substances were applied in 200 L water/ha. The substances were sprayed on bean leaves via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 23 °C - 27 °C; relative humidity: 62% - 73%; photoperiod: 16 hlight:8 h dark; light intensity: 1040 lux; food: larvae: *Sitotroga cerealella* eggs (UV-sterilized), adults: artificial diet.

Statistics: Descriptive statistics, Multiple Sequentially-rejective Fisher test after Bonferroni-Holm (α = 0.05) for mortality.

**II. RESULTS AND DISCUSSION**

A mortality of 6.0% was observed in the control group. In the test item treatment groups, mortalities ranged from 6.0 to 50.0% resulting in corrected mortality rates between 0% and 46.8%. Statistically significant effects on mortality were observed in the highest test item treatment group of 4.0 L product/ha (Multiple Sequentially-rejective Fisher test after Bonferroni-Holm, α = 0.05). No effects on reproduction of *Chrysoperla carnea* occurred, when the test item was applied at rates up to and including 4.0 L/ha BAS 736 00 F. Reproduction in the control and all test item treatments number of eggs per female per day was > 15 and the hatching rate was > 70% in the control. The results are summarized in Table A 30.

Table A 30: Effects on lacewings (*Chrysoperla carnea*) exposed to BAS 736 00 F in an extended

laboratory trial

| **Treatment** | **Rate  [L/ha]**1) | **Mortality  [%] 2)** | **Mortality corr.  [%] 3)** | **Reproduction [eggs/female/day]** | **Hatching rate [%]** |
| --- | --- | --- | --- | --- | --- |
| Control | -- | 6.0 | -- | 21.3 | 74.2 |
| BAS 736 00 F | 0.25 | 8.0 | 2.1 | 22.7 | 74.4 |
| 0.5 | 6.0 | 0 | 21.7 | 73.9 |
| 1.0 | 18.0 | 12.8 | 22.2 | 74.2 |
| 2.0 | 20.0 | 14.9 | 21.5 | 73.9 |
| 4.0 | 50.0 \* | 46.8 | 19.6 | 74.1 |
|  | **Endpoint [L BAS** **736 00 F/ha]** | | | | |
| LR50 | > 4.0 | | | | |
| ER50 4) | > 4.0 | | | | |

\* Statistically significant difference from the control (Multiple Sequentially-rejective Fisher test after Bonferroni-Holm, α = 0.05).

1) Application rate in 200 L water/ha.

2) Mortality: percentage of individuals, which did not reach maturity.

3) Corrected mortality according to Abbott (1925).

4) ER50 not given in the study report but estimated based on the raw data.

The reference item caused a mortality of 66.0% of exposed lacewings, resulting in a corrected mortality

of 63.8%.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to Vogt *et al.* (2000)** | **Obtained in this study** |
| Pre-imaginal mortality in the control group should not exceed 20% | 6.0% |
| Mean egg production in the control should be ≥ 15 eggs per female per day | 21.3 |
| Mean hatching rate of the eggs in the control should be ≥ 70% | 74.2% |
| Corrected mortality in the toxic reference treatment should be ≥ 50% | 63.8% |

All validity criteria were met.

**III. CONCLUSION**

**In an extended laboratory study with *Chrysoperla carnea*, the LR50 was > 4 L BAS 736 00 F/ha. No unacceptable effects on reproduction were observed up to and including an application rate of 4 L BAS 736 00 F/ha.**

* + 1. KCP 10.3.2.3 Semi-field studies with non-target arthropods

As BAS 736 00 F poses no unacceptable risk to non-target arthropods, further studies are not necessary.

* + 1. KCP 10.3.2.4 Field studies with non-target arthropods

As BAS 736 00 F poses no unacceptable risk to non-target arthropods, further studies are not necessary.

* + 1. KCP 10.3.2.5 Other routes of exposure for non-target arthropods

As BAS 736 00 F poses no unacceptable risk to non-target arthropods, further studies are not necessary.

* 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: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | CP 10.4.1.1/1 |
| Report | Effects of BAS 700 F (Fluxapyroxad) on reproduction and growth of earthworms Eisenia fetida in artificial soil,  Witte, B., 2014  report No EU-93491002,EU-704426,93491022  2014/1135449  Authority registration No |
| Guideline(s): | ISO 11268-2 (2012), OECD 222 - Earthworm reproduction Test (2004) |
| Deviations: | No |
| GLP: | yes  (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of BAS 700 F (Reg. No. 5 094 351, fluxapyroxad) on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Five test concentrations (30, 48, 76, 122 and 196 mg BAS 700 F/kg dry soil) were incorporated into the soil (containing 10% peat) with four replicates per treatment, each containing 10 worms. An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessments of worm mortality, body weight and feeding activity were carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.

BAS 700 F did not show any statistically significant effects on mortality and body weight, compared to the control. No mortality was observed at any test item concentration or in the control. The body weight changes were not statistically significantly different compared to the control in any test item treated group. The reproduction rates were not statistically different compared to the control up to and including 122 mg/kg dry soil. At the concentration of 196 mg BAS 700 F/kg dry soil the reproduction was statistically significantly reduced compared to the control. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

**In a 56-day earthworm reproduction study with BAS 700 F, no adverse effects on survival and biomass development could be determined at concentrations up to and including 196 mg/kg dry soil. The NOEC for mortality, biomass and feeding activity was determined to be equal or greater than 196 mg BAS 700 F/kg dry soil, the highest concentration tested. No adverse effects on reproduction could be determined at concentrations up to and including 122 mg BAS 700 F/kg dry soil. The NOEC for reproduction was equivalent to 122 mg BAS 700 F/kg dry soil.**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 700 F (Reg. No. 5 094 351, fluxapyroxad); batch no.: COD-001466; purity: 100% nominal (tolerance ± 1.0%).

**B. STUDY DESIGN**

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight range 304 to 596 mg), 9 -10 months old, source: in-house culture.

Test design: 56-day test in treated artificial soil prepared according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 worms each. Assessment of adult worm mortality, behavioral effects and biomass development was carried out after 28 days. Reproduction rate (number of offspring) was assessed after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, feeding activity and reproduction rate.

Reference item: Luxan Carbendazim 500 FC. The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 30, 48, 76, 122 and 196 mg BAS 700 F/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 5.7 – 6.0 at test initiation, pH 5.9 – 6.0 at test termination; water content 31.2% – 33.4% of maximum water holding capacity (WHC) at test initiation and 33.8% – 35.8% of the maximum WHC at test termination; temperature: 18 °C – 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 - 800 lux.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics, Williams t-test for weight change and reproduction (α = 0.05, two-sided).

**II. RESULTS AND DISCUSSION**

BAS 700 F did not show any statistically significant effects on mortality and body weight, compared to the control (Williams t-test, two-sided, α = 0.05). No mortality was observed at any test item concentration or in the control. The body weight changes were not statistically significantly different compared to the control in any test item treated group (Williams t-test, two-sided, α = 0.05). The reproduction rates were not statistically different compared to the control up to and including 122 mg/kg dry soil (Williams t-test, two-sided, α = 0.05). At the concentration of 196 mg BAS 700 F/kg dry soil the reproduction was statistically significantly reduced compared to the control (Williams t-test, two-sided, α = 0.05). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. The results are summarized in Table A 31.

**Table A 31: Effects of BAS 700 F on earthworms (*Eisenia fetida)* in a 56-day reproduction study**

| **BAS 700 F [mg/kg dry soil]** | **Control** | **30** | **48** | **76** | **122** | **196** |
| --- | --- | --- | --- | --- | --- | --- |
| Mortality (day 28) [%] | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Weight change (day 28) [%] | 41.9 | 39.0 | 38.3 | 42.5 | 48.1 | 47.9 |
| No. of juveniles (day 56) | 222 | 247 | 217 | 177 | 187 | 126 \* |
| Reproduction (day 56) [% of control] | -- | 111.5 | 97.7 | 79.8 | 84.2 | 56.6 |
| **Endpoints [mg/kg dry soil]** | | | | | | |
| NOEC (day 28) (mortality and weight) | ≥ 196 | | | | | |
| NOEC (day 56) (reproduction) | 122 | | | | | |

\* Statistically significantly different to control (Williams t-test, two-sided, α = 0.05).

In a separate study the reference item Luxan Carbendazim 500 FC had a significant effect reproduction of *Eisenia fetida* at a concentration of 1.30 mg carbendazim/kg dry soil. The EC50 for reproduction was calculated as 1.32 mg carbendazim/kg dry soil.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 222 (2016)** | **Obtained in this study** |
| Adult mortality in the control ≤ 10% | 0% |
| Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate) | 187 to 248 |
| Coefficient of variation of reproduction in the control ≤ 30% | 9.5% |

All validity criteria were met.

**III. CONCLUSION**

**In a 56-day earthworm reproduction study with BAS 700 F, no adverse effects on survival and biomass development could be determined at concentrations up to and including 196 mg/kg dry soil. The NOEC for mortality, biomass and feeding activity was determined to be equal or greater than 196 mg BAS 700 F/kg dry soil, the highest concentration tested. No adverse effects on reproduction could be determined at concentrations up to and including 122 mg BAS 700 F/kg dry soil. The NOEC for reproduction was equivalent to 122 mg BAS 700 F/kg dry soil.**

* + - * 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.4.1.1/2 |
| Report | Effects of BAS 736 00 F on the reproduction of the earthworm Eisenia andrei in artificial soil,  Friedrich, S., 2019  report No 849650, 1948TEC0047  2019/1061096  Authority registration No |
| Guideline(s): | OECD 222 (2016) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a chronic toxicity study, adults of *Eisenia andrei* (Annelida: Oligochaeta) were exposed to BAS 736 00 F. The test item was mixed into artificial soil (10% peat) at eight test concentrations of 20.0, 34.0, 57.8, 98.3, 167.0, 284.0, 482.8 and 820.7 mg BAS 736 00 F/kg dry soil with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure, no statistically significant effects on mortality and body weight were observed. The mortality of adult worms ranged between 0 – 7.5% in the test item treated groups and was 1.3% in the control group. The weight change of adult worms was 25.3 – 33.1% in the test item treated groups and 30.4% in the control group. The feeding activity in all test item treated groups was comparable to the control. The reproduction rate was statistically significantly different compared to the control at concentrations of 482.8 and 820.7 mg BAS 736 00 F/kg dry soil. No pathological symptoms and no further effects on behavior of the worms were observed.

**In a 56-day earthworm reproduction study with BAS 736 00 F, the NOEC for mortality and biomass was determined to be ≥ 820.7 mg/kg dry soil. The NOEC for reproduction was determined to be 284 mg/kg dry soil. The EC10, EC20 and EC50 values for reproduction were calculated to be 295.9, 401.4 and 719.3 mg/kg dry soil, respectively.**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Earthworm (*Eisenia andrei*), adult worms (with clitellum), weight: 274 – 430 mg/worm, age: approx. 4 months old; source: W. Neudorff GmbH KG, followed by in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test item are mixed homogeneously into the soil; 9 treatment groups were set up (8 test item concentrations, untreated control) with 4 replicates for the test item treatments and 8 replicates for the control, 10 worms per replicate. Assessment of worm mortality, behavioral effects, and biomass development after 28 days of exposure; after an additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

Endpoints: Mortality (LC50, NOEC), weight change (EC50, NOEC), feeding activity, reproduction rate (EC10, 20 and 50, NOEC).

Reference item: Maypon Flow (Carbendazim, SC 500). The effects of the reference item were investigated in a separate study.

Test concentrations: Control: untreated soil; test item: 20.0, 34.0, 57.8, 98.3, 167.0, 284.0, 482.8 and 820.7 mg BAS 736 00 F/kg dry soil, spacing factor: 1.7.

|  |  |  |  |
| --- | --- | --- | --- |
| BAS 736 00 F  [mg/kg dry soil] | Total active substances  [mg/kg dry soil]\* | BAS 9164 F  [mg/kg dry soil]\* | BAS 700 F  [mg/kg dry soil]\* |
| 20.0 | 2.32 | 1.39 | 0.93 |
| 34.0 | 3.94 | 2.37 | 1.58 |
| 57.8 | 6.70 | 4.02 | 2.68 |
| 98.3 | 11.4 | 6.84 | 4.56 |
| 167.0 | 19.4 | 11.62 | 7.75 |
| 284.0 | 32.9 | 19.76 | 13.17 |
| 482.8 | 56.0 | 33.59 | 22.39 |
| 820.7 | 95.2 | 57.10 | 38.07 |

\* The amounts of BAS 700 F and BAS 9164 F were calculated based on the nominal contents a.s. The density (1.078 g/cm³) was taken into account.

Test conditions: Artificial soil according to OECD 222 (10% peat); pH 5.87 – 6.03 at test initiation, 5.65 – 5.73 at test end; water content 55.7 – 56.0% of max. water holding capacity (WHC) at test start and 54.5 – 55.8% of WHC at test end; temperature: 19.4 °C – 21.6 °C; photoperiod: 16 hours light: 8 hours dark, light intensity: 630 lux, food: horse manure.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher test after Bonferroni-Holm (α = 0.05, one-sided greater) for mortality, Dunnett’s t-test for weight change and Williams t-test (α = 0.05, one-sided smaller) for reproduction; 3-parametric normal cumulative distribution function (CDF) for calculation of ECx.

**II. RESULTS AND DISCUSSION**

After 28 days of exposure, no statistically significant effects on mortality and body weight were observed. The mortality of adult worms ranged between 0 – 7.5% in the test item treated groups and was 1.3% in the control group. The weight change of adult worms was 25.3 – 33.1% in the test item treated groups and 30.4% in the control group. The feeding activity in all test item treated groups was comparable to the control. The reproduction rate was statistically significantly different compared to the control at concentrations of 482.8 and 820.7 mg BAS 736 00 F/kg dry soil (Williams-t-test, α = 0.05, one-sided smaller). No pathological symptoms and no further effects on behavior of the worms were observed. The results are summarized in Table A 32.

**Table A 32: Effects of BAS 736 00 F on *Eisenia andrei* in a 56-day reproduction study**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **BAS 736 00 F [mg/kg dry soil]** | **Control** | **20.0** | **34.0** | **57.8** | **98.3** | **167.0** | **284.0** | **482.8** | **820.7** |
| Mortality (28 d) [%] | 1.3 | 0.0 | 2.5 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 7.5 |
| Weight change (28 d) [%] | 30.4 | 31.3 | 33.1 | 30.5 | 29.6 | 29.3 | 30.2 | 31.9 | 25.3 |
| Number of juveniles (56 d) | 216.8 | 215.8 | 208.0 | 213.3 | 217.8 | 212.3 | 185.3 | 162.3 \* | 87.5 \* |
| Coefficient of variation [%] | 13.5 | 14.1 | 11.0 | 17.4 | 16.6 | 16.9 | 13.3 | 9.3 | 25.5 |
| Reproduction [% of control] (56 d) | 100 | 99.5 | 96.0 | 98.4 | 100.5 | 97.9 | 85.5 | 74.9 | 40.4 |
|  | **Endpoints [mg BAS 736 00 F/kg dry soil]** | | | | | | | | |
| NOEC(28 d) | ≥ 820.7 | | | | | | | | |
| NOEC(56 d) | 284.0 | | | | | | | | |
| LC50 (28 d) 1 | > 820.7 | | | | | | | | |
| EC10 (56 d) 2 | 295.9  (95% confidence limits 243.2 – 355.5) | | | | | | | | |
| EC20 (56 d) 2 | 401.4  (95% confidence limits 350.3 – 457.2) | | | | | | | | |
| EC50 (56 d) 2 | 719.3  (95% confidence limits 670.6 – 771.6) | | | | | | | | |

\* statistically significantly different from control (Williams-t-test for reproduction, α = 0.05, one-sided smaller)

1 based on estimation of the data

2 based on 3-parametric normal CDF

In a separate study, the reference item Maypon Flow (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of earthworms. The reproduction rate was clearly inhibited by 58% and 99% compared to the control at the tested concentrations of 5 and 10 mg product/kg dry soil.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 222 (2016)** | **Obtained in this study** |
| Adult mortality in the control ≤ 10% | 1.3% |
| Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate) | 168 to 253 |
| Coefficient of variation of reproduction in the control ≤ 30% | 13.5% |

All validity criteria were met.

**III. CONCLUSION**

**In a 56-day earthworm reproduction study with BAS 736 00 F, the NOEC for mortality and biomass was determined to be ≥ 820.7 mg/kg dry soil. The NOEC for reproduction was determined to be 284 mg/kg dry soil. The EC10, EC20 and EC50 values for reproduction were calculated to be 295.9, 401.4 and 719.3 mg/kg dry soil, respectively.**

* + - 1. KCP 10.4.1.2 Earthworms - field studies

As BAS 736 00 F poses no unacceptable risk to earthworms, further studies are not necessary.

* + 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: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | CP 10.4.2.1/1 |
| Report | Effects of BAS 700 F (Fluxapyroxad) on the reproduction of the collembolan Folsomia candida,  Friedrich, S., 2014  report No EU-141048070S,EU-706151,14 10 48 070 S  2014/1135433  Authority registration No |
| Guideline(s): | ISO 11267 (1999), OECD 232 (2009) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of BAS 700 F on mortality and reproduction of collembola *(Folsomia candida)* were investigated in a laboratory study over 28 days. Five application rates (31.25, 62.5, 125, 250 and 500 mg BAS 700 F/kg dry soil) were incorporated into the soil with 4 replicates per treatment (each containing 10 collembolans). A solvent control with 8 replicates was included. Assessments of adult springtail mortality, behavioral effects and reproduction rate (number of juveniles) was carried out after 28 days.

After 28 days of exposure, no statistically significant mortality was observed in the test item groups compared to the control. Mortality rates of 0 - 5.0% were recorded in the test item treatment groups. In the solvent control the mortality rate was 2.5%. The reproduction was not statistically significant different compare to the control in the test item groups. The mean reproduction in the solvent control reached 852 juveniles. Reproduction rates in 31.25, 62.5, 125, 250 and 500 mg BAS 700 F/kg soil dry weight were 847, 829, 854, 857 and 890 juveniles, respectively.

**In a 28-day collembola reproduction study with BAS 700 F the NOEC based on mortality and reproduction was ≥ 500 mg BAS 700 F/kg dry soil. The EC10 was estimated to be > 500 mg BAS 700 F/kg dry soil.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 700 F (fluxapyroxad, Reg. No. 5 096 351), batch COD-001466; purity: 100% analyzed (tolerance ±1.0%).

**B. STUDY DESIGN**

Test species: Collembola (*Folsomia candida)*, juveniles (9 – 12 days old); source: in-house culture.

Test design: 28-day test in treated artificial soil according to OECD 232 (5% peat); artificial soil filled in glass vessels was treated with different concentrations of the test item before collembolans were introduced; 6 treatment groups (5 test item concentrations, solvent control); 4 replicates for each test item treatment and 8 replicates for the solvent control, each with 10 collembolans. Feeding of collembolans with about 2 mg dry yeast at the beginning of the test for each test vessel and on day 14. Assessments of adult collembolan mortality, behavioral effects and reproduction rate (number of juveniles) after 28 days.

Endpoints: Mortality, reproduction rate.

Test rates: Solvent control, 31.25, 62.5, 125, 250 and 500 mg BAS 700 F/kg dry soil (based on analyzed content).

Reference item: Boric acid (100% analyzed). The effects of the reference item were investigated in a separate study.

Test conditions: Artificial soil according to OECD 232 (5% peat); pH 5.98 – 6.05 at test initiation, pH 5.79 – 5.87 at test termination; water content at study initiation 57.7 – 58.0% of maximum water holding capacity (WHC) and 56.4 – 57.3% of maximum WHC at test termination; temperature: 18.0 °C – 21.9 °C; photoperiod: 16 h light: 8 h dark, light intensity: 470 lux; food: 2 mg dry yeast at the start of the test and after 14 days.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics, Fisher’s Exact Binomial Test with Bonferroni Correction for mortality (one-sided greater, α = 0.05), Williams-t-test for reproduction (one-sided smaller, α = 0.05).

**II. RESULTS AND DISCUSSION**

After 28 days of exposure, no statistically significant mortality was observed in the test item groups compared to the control (Fisher’s Exact Binomial Test, one-sided greater, α = 0.05). Mortality rates of 0 - 5.0% were recorded in the test item treatment groups. In the solvent control the mortality rate was 2.5%. The reproduction was not statistically significant different compared to the control in the test item groups (Williams-t-test, one-sided smaller, α = 0.05). The mean reproduction in the solvent control reached 852 juveniles. Reproduction rates in 31.25, 62.5, 125, 250 and 500 mg/kg soil dry weight were 847, 829, 854, 857 and 890 juveniles, respectively. The results are summarized in Table A 33.

**Table A 33: Effects of BAS 700 F on collembola (*Folsomia candida)* in a 28-day reproduction**

**study**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **BAS 700 F [mg/kg dry soil]** | **Solvent control** | **31.25** | **62.5** | **125.0** | **250.0** | **500.0** |
| Mortality (day 28) [%] | 2.5 | 2.5 | 5.0 | 2.5 | 0.0 | 2.5 |
| No. of juveniles (day 28) | 852 | 847 | 829 | 854 | 857 | 890 |
| Reproduction in [%] of control (day 28) | 100 | 99 | 97 | 100 | 101 | 104 |
|  | **Endpoints [mg BAS 700 F/kg dry soil]** | | | | | |
| NOEC (day 28) | ≥ 500 | | | | | |
| LC50 1) | > 500 | | | | | |
| EC10, EC20, EC50 1) | > 500 | | | | | |

1) based on estimation of the data.

The EC50 (reproduction) of the reference item boric acid was calculated to be 104 mg BAS 700 F/kg soil dry weight.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 232 (2016)** | **Obtained in this study** |
| Mean adult mortality in the control ≤ 20% | 2.5% (Solvent control) |
| Mean number of juveniles per control replicate ≥ 100 | 852 |
| Coefficient of variation of reproduction in the control ≤ 30% | 13.6% |

All validity criteria were met.

**III. CONCLUSION**

**In a 28-day collembolan reproduction study with BAS 700 F the NOEC based on mortality and reproduction was ≥ 500 mg BAS 700 F/kg dry soil. The EC10 was estimated to be > 500 mg BAS 700 F/kg dry soil.**

* + - * 1. Study 2

|  |  |
| --- | --- |
| Comments of zRMS: | Study not evaluated. |

|  |  |
| --- | --- |
| Reference: | CP 10.4.2.1/2 |
| Report | Effects of BAS 700 F (Fluxapyroxad) on the reproduction of the predatory mite Hypoaspis aculeifer,  Schulz, L., 2016  report No EU-799167,16 10 48 229 S  2016/1122885  Authority registration No |
| Guideline(s): | OECD 226 (2008) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of BAS 700 F on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. Eight concentrations (16.3, 29.4, 52.9, 95.3, 171.5, 308.7, 555.6 and 1000 mg BAS 700 F/kg dry soil) were incorporated into the soil. For the solvent control, 5 mL acetone was added, but otherwise the soil was left untreated. Eight replicates and 4 replicates were prepared for the solvent control and test item treatment groups, respectively, each containing 10 adult soil mites (females). Assessment of adult mortality and reproduction effects was carried out after 14 days of exposure.

Mortality rates of 0.0% - 5.0% were recorded in the test item treatment groups. In the solvent control group, the mortality rate was 6.3%. The observed mortality rates for adult mortality in the test item treatment groups compared to solvent control were not statistically significant. Differences in behavior and morphology of the mites between the solvent control and the test item treatment groups could not be observed. Reproduction rates in the 16.3, 29.4, 52.9, 95.3, 171.5, 308.7, 555.6 and 1000 mg BAS 700 F/kg dry soil were 309.8, 263.3, 276.5, 272.5, 276.8, 284.0, 298.8 and 299.3 juveniles, respectively. The mean reproduction in the solvent control reached 301.4 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations compared to solvent control.

**In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 700 F, the LC50, EC10, EC20 and EC50 are estimated to be higher than 1000 mg BAS 700 F/kg dry soil, the highest concentration tested. The NOEC for mortality and for reproduction was determined to be ≥ 1000 mg 700 F/kg dry soil, the highest concentration tested.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 700 F (fluxapyroxad); batch no. COD-001466, analyzed purity: 100.0% (± 1.0%).

**B. STUDY DESIGN**

Test species: Predatory soil mites: *Hypoaspis aculeifer* (Canestrini*)*, adult females from a synchronized culture with an age difference of 2 days; source: in-house culture.

Test design: 14-day chronic laboratory test in treated artificial soil according to OECD 226. Different concentrations of the test item were mixed homogenously into artificial soil and used to fill glass vessels after which mites were introduced on top of the soil; Nine treatment groups (eight test item concentrations, solvent control); 4 replicates for each test item treatment and 8 replicates for the control group, each containing 10 mites. Feeding of mites with *Tyrophagus putrescentiae* (Schrank) at the beginning and *ad libitum* during the test. Assessments of adult mortality and reproduction effects were carried out after 14 days of exposure.

Endpoints: Mortality, reproduction rate (no. of juveniles) after 14 days of exposure.

Reference item: Dimethoate (EC 400 g/L nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Solvent control (acetone); test item: 16.3, 29.4, 52.9, 95.3, 171.5, 308.7, 555.6, 1000 mg BAS 700 F/kg soil dry (spacing factor: 1.8).

Test conditions: Artificial soil according to OECD 226; pH 5.9 – pH 6.1 at test initiation, pH 5.5 + pH 5.7 at test termination; water content at study initiation 44.61% – 49.17% of maximum water holding capacity (WHC) and 42.37% –45.86% of maximum WHC at test termination; temperature: 19.7 °C – 21.0°C; light conditions: artificial light (Lumilux L58W), intensity: 508 lx, photoperiod: 16 h light : 8 h dark.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Fisher’s Exact Binominal Test with Bonferroni correction for mortality (one-sided greater, α = 0.05), Dunnett-t-test for reproduction data (one-sided smaller, α = 0.05).

**II. RESULTS AND DISCUSSION**

Mortality rates of 0.0% - 5.0% were recorded in the test item treatment groups. In the solvent control group, the mortality rate was 6.3%. The observed mortality rates for adult mortality in the test item treatment groups compared to solvent control were not statistically significant (Fisher’s Exact Binomial Test with Bonferroni correction, α = 0.05, one-sided greater). Differences in behavior and morphology of the mites between the solvent control and the test item treatment groups could not be observed. Reproduction rates in the 16.3, 29.4, 52.9, 95.3, 171.5, 308.7, 555.6 and 1000 mg BAS 700 F/kg dry soil were 309.8, 263.3, 276.5, 272.5, 276.8, 284.0, 298.8 and 299.3 juveniles, respectively. The mean reproduction in the solvent control reached 301.4 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations compared to solvent control (Dunnett-t-test, α = 0.05, one-sided smaller. The results are summarized in Table A 34.

**Table A 34: Effects of BAS 700 F on predatory mite (*Hypoaspis aculeifer)* in a 14-day**

**reproduction study**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **BAS 700 F [mg/kg dry soil]** | **Solvent control** | **16.3** | **29.4** | **52.9** | **95.3** | **171.5** | **308.7** | **555.6** | **1000** |
| Mean adult mortality [%] | 6.3 | 2.5 | 2.5 | 5.0 | 5.0 | 0.0 | 2.5 | 5.0 | 2.5 |
| Mean number of juveniles (day 14) | 301.4 | 309.8 | 263.3 | 276.5 | 272.5 | 276.8 | 284.0 | 298.8 | 299.3 |
| Reproduction in [%] of solvent control (day 14) | 100 | 103 | 87 | 92 | 90 | 92 | 94 | 99 | 99 |
| **Endpoints** | **[mg BAS 700 F/kg dry soil]** | | | | | | | | |
| NOEC (mortality, reproduction) | ≥ 1000 | | | | | | | | |
| LC50 1) | > 1000 | | | | | | | | |
| EC10, EC20, EC50 1) | > 1000 | | | | | | | | |

1) Based on estimation.

In a separate study, the EC50 reproduction of the reference item was calculated to be 3.1 mg dimethoate/kg dry soil.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 226 (2016)** | **Obtained in this study** |
| Mean adult mortality in the control ≤ 20% | 6.3% (solvent control) |
| Mean number of juveniles per control replicate ≥ 50 | 301.4 |
| Coefficient of variation of reproduction in the control ≤ 30% | 13.7% |

All validity criteria were met.

**III. CONCLUSION**

**In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 700 F, the LC50, EC10, EC20 and EC50 are estimated to be higher than 1000 mg BAS 700 F/kg dry soil, the highest concentration tested. The NOEC for mortality and for reproduction was determined to be ≥ 1000 mg BAS 700 F/kg dry soil, the highest concentration tested.**

* + - * 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.4.2.1/3 |
| Report | Effects of BAS 736 00 F on the reproduction of the collembolan Folsomia candida,  Friedrich, S., 2019  report No 849653, 1948TCC0036  2019/1061097  Authority registration No |
| Guideline(s): | OECD 232 |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of BAS 736 00 F on survival and reproduction of the collembolan *Folsomia candida* were investigated in a laboratory study over 28 days. The test item was mixed into artificial soil (5% peat) at concentrations of 30, 51, 87, 147, 251, 426, 724, 1231 mg BAS 736 00 F/kg dry soil with 4 replicates per treatment. An untreated control with 8 replicates was included. Each replicate contained 10 juvenile collembolans. Assessment of adult mortality and reproduction (number of juveniles) was carried out after 28 days.

After 28 days of exposure, mortalities ranging from 0.0% to 100.0% were observed in the test item treatments compared to 2.5% in the control. Statistically significant differences on mortality were observed at concentrations of 426, 724 and 1231 mg BAS 736 00 F/kg dry soil. In the control, a mean of 1342 juveniles were counted. In the treatment groups, a mean number of juveniles between 43 to 1368 was counted. This is corresponding to a reproduction relative to the control between 3.2% and 102.0%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 251 mg BAS 736 00 F/kg dry soil and above. No behavioral abnormalities were observed at any tested concentration.

**In a 28-day collembolan reproduction study with BAS 736 00 F, the LC50 was determined to be 529 mg/kg dry soil and the NOEC for mortality was 251 mg/kg dry soil. For reproduction, the EC10 was determined to be 232 mg/kg dry soil and the NOEC was determined to be 147 mg/kg dry soil.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Collembola (*Folsomia candida*), juveniles, 9 - 12 days old; source: in-house culture.

Test design: 28-day exposure in treated artificial soil (5% peat); different concentrations of the test item were mixed homogeneously into the soil, then filled in glass vessels where springtails were introduced on top of the soil surface; 9 treatment groups (8 concentrations of the test item, untreated control) with 4 replicates for the test item treatment groups and 8 replicates for the control, with 10 collembolans per replicate. Assessment of adult mortality, behavioral effects and reproduction (number of juveniles) after 28 days.

Endpoints: Mortality (NOEC, LC50) and reproduction rate (NOEC, EC10, 20 and 50) after 28 days.

Reference item: Boric acid - the effects of the reference item were investigated in a separate study.

Test concentrations: Control: untreated soil, test item: 30, 51, 87, 147, 251, 426, 724, 1231 mg BAS 736 00 F/kg dry soil, spacing factor: 1.7.

|  |  |  |  |
| --- | --- | --- | --- |
| BAS 736 00 F  [mg/kg dry soil] | Total active substances  [mg/kg dry soil]\* | BAS 9164 F  [mg/kg dry soil]\* | BAS 700 F  [mg/kg dry soil]\* |
| 30 | 3.5 | 2.09 | 1.39 |
| 51 | 5.9 | 3.55 | 2.37 |
| 87 | 10 | 6.0 | 4.0 |
| 147 | 17 | 10.3 | 6.8 |
| 251 | 29 | 17.4 | 11.6 |
| 426 | 49 | 29.6 | 19.8 |
| 724 | 84 | 50.4 | 33.6 |
| 1231 | 143 | 85.6 | 57.1 |

\* The amounts of BAS 700 F and BAS 9164 F were calculated based on the nominal contents a.s. The density (1.078 g/cm³) was taken into account.

Test conditions: Artificial soil according to OECD 232 (5% peat); pH 6.00 - 6.02 at test initiation and pH 5.70 – 5.78 at test termination; water content at test start 58.6 – 59.1% of maximum water holding capacity (WHC) and at test end 56.9 – 58.4% of maximum WHC; temperature: 19.4 – 21.6 °C; photoperiod: 16 h light : 8 h dark, light intensity: 600 lux; food: approx. 2 mg granulated dry yeast at the start of the test and after 14 days.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Step-down Cochran-Armitage test for mortality (one-sided greater, α = 0.05), Williams-t-test for reproduction (one-sided smaller, α = 0.05), Weibull analysis for mortality, 3-parametric normal cumulative distribution function (CDF) for reproduction.

**II. RESULTS AND DISCUSSION**

After 28 days of exposure, mortalities ranging from 0.0% to 100.0% were observed in the test item treatments compared to 2.5% in the control. Statistically significant differences on mortality were observed at concentrations of 426, 724 and 1231 mg BAS 736 00 F /kg dry soil (Step-down Cochran-Armitage test, α = 0.05, one-sided greater).

In the control, a mean of 1342 juveniles were counted. In the treatment groups, a mean number of juveniles between 43 to 1368 was counted. This is corresponding to a reproduction relative to the control between 3.2% and 102.0%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 251 mg BAS 736 00 F/kg dry soil and above (Williams-t-test, one-sided smaller, α = 0.05). No behavioral abnormalities were observed at any tested concentration. The results are summarized in Table A 35.

**Table A 35: Effects of BAS 736 00 F on *Folsomia candida* in a 28-day reproduction study**

| **BAS 736 00 F [mg/kg dry soil]** | **Control** | **30** | **51** | **87** | **147** | **251** | **426** | **724** | **1231** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Mortality (day 28) [%] | 2.5 | 0.0 | 5.0 | 2.5 | 5.0 | 5.0 | 37.5\* | 80.0\* | 100.0\* |
| No. of juveniles (day 28) | 1342 | 1368 | 1296 | 1376 | 1263 | 1096\* | 884\* | 356\* | 43\* |
| Coefficient of variation [%] | 11.5 | 15.0 | 8.7 | 7.7 | 1.8 | 19.0 | 18.5 | 41.2 | 55.1 |
| Reproduction [% of control] (day 28) | 100 | 102.0 | 96.6 | 102.5 | 94.1 | 81.7 | 65.9 | 26.5 | 3.2 |
|  | **Endpoints [mg BAS 736 00 F/kg dry soil]** | | | | | | | | |
| NOECmortality | 251 | | | | | | | | |
| NOECreproduction | 147 | | | | | | | | |
| LC50 1  (95% confidence limits) | 529  (413 – 677) | | | | | | | | |
| EC10 2  (95% confidence limits) | 232  (219 – 246) | | | | | | | | |
| EC20 2  (95% confidence limits) | 303  (290 – 317) | | | | | | | | |
| EC50 2  (95% confidence limits) | 505  (493 – 518) | | | | | | | | |

\* Statistically significant differences compared to the control (Step-down Cochran-Armitage test for mortality, one-sided greater, α = 0.05; Williams-t-test for reproduction; α = 0.05, one-sided smaller).

1 based on Weibull analysis

2 based on 3-parametric normal CDF

In a separate study, the EC50 (reproduction) of the reference item boric acid was calculated to be 98 mg/kg dry soil. The results of the reference test demonstrate the sensitivity of the test system.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 232 (2016)** | **Obtained in this study** |
| Mean adult mortality in the control ≤ 20% | 2.5% |
| Mean number of juveniles per control replicate ≥ 100 | 1342 |
| Coefficient of variation of reproduction in the control ≤ 30% | 11.5% |

All validity criteria were met.

**III. CONCLUSION**

**In a 28-day collembolan reproduction study with BAS 736 00 F, the LC50 was determined to be 529 mg/kg dry soil and the NOEC for mortality was 251 mg/kg dry soil. For reproduction, the EC10 was determined to be 232 mg/kg dry soil and the NOEC was determined to be 147 mg/kg dry soil.**

* + - * 1. Study 4

|  |  |
| --- | --- |
| 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. |

|  |  |
| --- | --- |
| Reference: | CP 10.4.2.1/4 |
| Report | Effects of BAS 736 00 F on the reproduction of the predatory mite Hypoaspis aculeifer,  Schulz, L., 2021  report No 849655, 1948THC0030  2019/1061098  Authority registration No |
| Guideline(s): | OECD 226 (2016) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

The effects of BAS 736 00 F on mortality and reproduction of the soil mite *(Hypoaspis aculeifer)* were investigated in a laboratory study over 14 days. The test item was mixed into artificial soil (5% peat) at rates of 74.51, 119.2, 190.7, 305.2, 488.3, 781.3, 1250 and 2000 mg BAS 736 00 F/kg dry soil, with 4 replicates per treatment. An untreated control with 8 replicates was included. Each replicate contained 10 adult mites. Assessment of adult mortality and reproduction (number of juveniles) was carried out after 14 days.

After 14 days of exposure, mortality rates ranging from 2.5% to 52.5% were observed in the test item treatments compared to 1.3% in the control. Statistically significant differences on mortality were observed at concentrations of 781.3, 1250 and 2000 mg BAS 736 00 F/kg dry soil. Differences in the behavior of the mites in the control and the test item treatments groups were not observed. In the control, a mean of 332.5 juveniles was counted. In the treatment groups, mean numbers of juveniles between 9.5 and 317.0 were counted. This is corresponding to a reproduction relative to the control ranging from 3% and 95%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 305.2 mg BAS 736 00 F/kg dry soil and above.

**In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 736 00 F, the LC50 value for mortality was calculated to be 2470.9 mg/kg dry soil. The EC10 value for reproduction was calculated to be 438.9 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be 488.3 and 190.7 mg/kg dry soil, respectively.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Soil mite (*Hypoaspis aculeifer* Canestrini), adult mites with an age difference of 2 days; source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of BAS 736 00 F on mortality and reproduction of soil mites. Different concentrations of the test item were homogenously mixed into artificial soil (5% peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 9 treatment groups (8 test item concentrations, 1 control); 8 replicates/control group and 4 replicates/test item treatments, each with 10 soil mites; Assessment of adult mortality and reproduction effects (number of juveniles) after 14 days

Endpoints: Mortality (NOEC, LC50) and reproduction rate (NOEC, EC10, 20 and 50) after 28 days.

Reference item: Dimethoate (98.8% ± 0.5%, analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Control, 74.51, 119.2, 190.7, 305.2, 488.3, 781.3, 1250 and 2000 mg BAS 736 00 F/kg dry soil (spacing factor: 1.6).

|  |  |  |  |
| --- | --- | --- | --- |
| BAS 736 00 F  [mg/kg dry soil] | Total active substances  [mg/kg dry soil]\* | BAS 9164 F  [mg/kg dry soil]\* | BAS 700 F  [mg/kg dry soil]\* |
| 74.51 | 8.6 | 5.2 | 3.5 |
| 119.2 | 13.8 | 8.3 | 5.5 |
| 190.7 | 22.1 | 13.3 | 8.8 |
| 305.2 | 35.4 | 21.2 | 14.2 |
| 488.3 | 56.6 | 34.0 | 22.6 |
| 781.3 | 90.6 | 54.4 | 36.2 |
| 1250 | 144.9 | 87.0 | 58.0 |
| 2000 | 231.9 | 139.1 | 92.8 |

\* based on nominal content of active substance and a test item density of 1.078 g/cm³, calculations were done with unrounded values.

Test conditions: Artificial soil according to OECD 226 (5% peat); pH 5.8 - 6.3 at test initiation, pH 5.3 - 5.4 at test termination; water content 46.67% - 49.16% of max. water holding capacity (WHC) at test initiation and at test termination 42.83% – 46.04% of max. WHC; temperature: 20.0 – 21.2 °C; photoperiod: 16 h light : 8 h dark, light intensity: 492 lux; food: cheese mite (*Tyrophagus putrescentiae*) supplied twice to three times a week.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics, Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm α = 0.05, one-sided greater) for mortality, Welch-t-test after Bonferroni-Holm for reproduction (α = 0.05, one-sided smaller), Weibull analysis using linear maximum likelihood regression for LCx determination, Non-linear regression analysis (3-parametric normal cumulative distribution function (CDF)) for ECx determination.

**II. RESULTS AND DISCUSSION**

After 14 days of exposure, mortality rates ranging from 2.5% to 52.5% were observed in the test item treatments compared to 1.3% in the control. Statistically significant differences on mortality were observed at concentrations of 781.3, 1250mand 2000 mg BAS 736 00 F/kg dry soil (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater). Differences in the behavior of the mites in the control and the test item treatments groups were not observed. In the control, a mean of 332.5 juveniles was counted. In the treatment groups, mean numbers of juveniles between 9.5 and 317.0 were counted. This is corresponding to a reproduction relative to the control ranging from 3% and 95%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 305.2 mg BAS 736 00 F/kg dry soil and above (Welch-t-test after Bonferroni-Holm, α = 0.05, one-sided smaller). The results are summarized in Table A 36.

**Table A 36: Effects of BAS 736 00 F on soil mite (*Hypoaspis aculeifer*) in a 14-day reproduction**

**study**

| **BAS 736 00 F [mg/kg dry soil]** | **Control** | **74.51** | **119.2** | **190.7** | **305.2** | **488.3** | **781.3** | **1250** | **2000** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Mortality (day 14) [%] | 1.3 | 5.0 | 5.0 | 5.0 | 2.5 | 12.5 | 15.0\* | 17.5\* | 52.5\* |
| No. of juveniles (day 14) | 332.5 | 300.5 | 317.0 | 307.8 | 298.3\* | 278.8\* | 239.8\* | 37.8\* | 9.5\* |
| Coefficient of variation [%] | 2.5 | 6.6 | 4.8 | 10.8 | 2.6 | 7.5 | 16.2 | 30.9 | 79.0 |
| Reproduction [% of control] (day 14) | 100 | 90 | 95 | 93 | 90 | 84 | 72 | 11 | 3 |
|  | **Endpoints [mg BAS 736 00 F/kg dry soil]** | | | | | | | | |
| NOECmortality | 488.3 | | | | | | | | |
| NOECreproduction | 190.7 | | | | | | | | |
| LC50 1  (95% confidence limits) | 2470.9  (1669.6 - 3656.8) | | | | | | | | |
| EC10 2  (95% confidence limits) | 438.9  (438.2 - 439.6) | | | | | | | | |
| EC20 2  (95% confidence limits) | 539.8  (538.9 - 540.6) | | | | | | | | |
| EC50 2  (95% confidence limits) | 801.8  (800.4 - 803.2) | | | | | | | | |

\* statistically significant compared to control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, α = 0.05, one-sided greater and Welch-t-test after Bonferroni-Holm for reproduction, α = 0.05, one-sided smaller)

1 10.5/1

2 based on 3-parameter normal CDF

In a separate study, the EC50 (reproduction) of the reference item dimethoate (98.8% ± 0.5%, analyzed) was calculated to be 4.4 mg a.s./kg dry soil. The results of the reference test demonstrate the sensitivity of the test system.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 226 (2016)** | **Obtained in this study** |
| Mean adult mortality in the control ≤ 20% | 1.3% |
| Mean number of juveniles per control replicate ≥ 50 | 332.5 |
| Coefficient of variation of reproduction in the control ≤ 30% | 2.5% |

All validity criteria were met.

**III. CONCLUSION**

**In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 736 00 F, the LC50 value for mortality was calculated to be 2470.9 mg/kg dry soil. The EC10 value for reproduction was calculated to be 438.9 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be 488.3 and 190.7 mg/kg dry soil, respectively.**

* + - 1. KCP 10.4.2.2 Higher tier testing

As BAS 736 00 F poses no unacceptable risk to other non-target soil macro-organisms, further studies are not necessary.

* 1. KCP 10.5 Effects on soil nitrogen transformation
     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. |

|  |  |
| --- | --- |
| Reference: | CP 10.5/1 |
| Report | Effects of BAS 736 00 F on the activity of soil microflora (Nitrogen transformation test),  Persdorf, M., 2019  report No 849649, 1948SMN0043  2019/1061099  Authority registration No |
| Guideline(s): | OECD 216 (2000) |
| Deviations: | No |
| GLP: | yes  (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a soil microbial activity study, the effects of BAS 736 00 F on nitrogen transformation were tested in silty-loamy sand soil. BAS 736 00 F was applied to samples of the soil at nominal concentrations of 7.00 mg and 35.00 mg/kg dry soil. Triplicate samples of each treatment were removed for analysis of NH4-nitrogen and NO3-nitrogen 0, 7, 14 and 28 days after application.

No unacceptable effects of BAS 736 00 F on nitrogen transformation in soil were observed at both test concentrations (7.00 mg and 35.00 mg/kg dry soil) after 28 days. Only negligible deviations from the control of -7.7% and +0.4% (at test concentration 7.00 mg and 35.00 mg/kg dry soil, respectively) were observed in both treatment groups after 28 days.

**Based on the results of this study, BAS 736 00 F caused no unacceptable short term and no long-term effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO3-N production) in a loamy sand soil tested up to a concentration of 35.00 mg/kg dry soil.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test soil: Biologically active agricultural soil: silty-loamy sand (DIN 4220) / sandy loam (USDA), soil pH 6.0, Corg 1.48%, WHC: 37.37%.

Test design: Determination of the N-transformation (NO3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Three treatment groups were set up (untreated control, 2 test item concentrations) with 3 replicates per treatment. Comparison of test item treated soil with a non-treated soil. NH4-nitrogen formed from organically bound nitrogen and NO3-nitrogen from the nitrification process was determined by using an Autoanalyzer (SEAL Analytical). Sampling scheme: 0, 7, 14 and 28 days after treatment; sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO3-nitrogen production 0, 7, 14 and 28 days after exposure.

Test rates: Control, 7.00 mg and 35.00 mg BAS 736 00 F/kg dry soil.

Reference item: Dinoterb (purity: 99.28% (g/g) analyzed). The reference item was tested in a separate study at rates 6.80, 13.60 and 27.20 mg/kg dry soil.

Test conditions: Water content: approx. 45% of its maximum water holding capacity; measured water content: 16.03% - 16.48%; pH 6.0 - 6.1. Soil samples were incubated at 19.1 °C - 20.5 °C while stored in glass flasks in the dark.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics.

**II. RESULTS AND DISCUSSION**

No unacceptable effects of BAS 736 00 F on nitrogen transformation in soil were observed at both test concentrations (7.00 mg and 35.00 mg/kg dry soil) after 28 days. Only negligible deviations from the control of -7.7% and +0.4% (at test concentration 7.00 mg and 35.00 mg/kg dry soil, respectively) were observed in both treatment groups after 28 days. The results are summarized below in Table A 37.‎

Table A 37: Effects of BAS 736 00 F on soil micro-organisms (nitrogen transformation rate) for

the intervals 0 - 7, 0 - 14 and 0 - 28

| **Soil (interval)** | **Control** | **7.00 mg BAS 736 00 F/kg dry soil** | | **35.00 mg BAS 736 00 F/kg dry soil** | |
| --- | --- | --- | --- | --- | --- |
| **NO3-N [mg/kg dry soil] 1** | **NO3-N [mg/kg dry soil] 1** | **% Deviation from control** | **NO3-N [mg/kg dry soil] 1** | **% Deviation from control** |
| Loamy sand  (0 - 7 d) | 40.20 | 36.23 | -9.9 | 44.23 | +10.0 |
| Loamy sand  (0 - 14 d) | 54.47 | 53.67 | -1.5 | 54.33 | -0.2 |
| Loamy sand (0 - 28 d) | 73.17 | 67.53 | -7.7 | 73.47 | +0.4 |
|  | **NO3-N/day [mg/kg dry soil/d] 2** | **NO3-N/day [mg/kg dry soil/d] 2** | **% Deviation from control** | **NO3-N/day [mg/kg dry soil/d] 2** | **% Deviation from control** |
| Loamy sand  (0 - 7 d) | 5.74 | 5.18 | -9.9 | 6.32 | +10.0 |
| Loamy sand  (0 - 14 d) | 3.89 | 3.83 | -1.5 | 3.88 | -0.2 |
| Loamy sand (0 - 28 d) | 2.61 | 2.41 | -7.7 | 2.62 | +0.4 |

1 Measured values sampling day “x” - measured values sampling day 0, mean of 3 replicates.

2 Daily rates are not included in the study report but are calculated based on the results.

- = inhibition, + = stimulation.



In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of

+34.2% at 13.60 mg/kg dry soil determined 28 days after application.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 216 (2000)** | **Obtained in this study** |
| Coefficient of variation in the control for NO3-N ≤ 15% | max. 6.3% (silty-loamy sand) |

All validity criteria were met.

**III. CONCLUSION**

**Based on the results of this study, BAS 736 00 F caused no unacceptable short term and no long-term effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO3-N production) in a loamy sand soil tested up to a concentration of 35.00 mg/kg dry soil.**

* 1. KCP 10.6 Effects on terrestrial non-target higher plants
     1. KCP 10.6.1 Summary of screening data

Tests on non-target plants have been conducted. The data point is covered by Appendix 2.6.2 (KCP 10.6.2).

* + 1. KCP 10.6.2 Testing on non-target plants
       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. |

|  |  |
| --- | --- |
| Reference: | CP 10.6.2/1 |
| Report | Effect of BAS 736 00 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions,  Maleck, A., 2019  report No 849658, AC/BASF/19/20  2019/1061112  Authority registration No |
| Guideline(s): | EPA 850.4150, OECD 227 July 2006 |
| Deviations: | No |
| GLP: | yes  (certified by Land Brandenburg Ministerium der Justiz und fuer Europa und fuer Verbraucherschutz, Potsdam, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a vegetative vigor test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean and tomato) and four species of monocotyledonous plants (onion, ryegrass, wheat and corn) were exposed to BAS 736 00 F to evaluate the phytotoxic potential. BAS 736 00 F was applied post-emergence at BBCH 12 to 14 at a limit rate of 2.0 L BAS 736 00 F/ha. After application, the plants were cultivated for 21 days under greenhouse conditions. Assessment of plant damage (phytotoxicity) and plant survival were done 7, 14 and 21 days after treatment (DAT). Assessments of plant length and plant dry weight were done at study termination 21 DAT.

After exposure, no plant mortality was observed for all tested plant species following the application of 2.0 L BAS 736 00 F/ha at BBCH stage 12-14. No symptoms of phytotoxicity were observed for all plant species, except for oilseed rape and soybean. Oilseed rape und soybean showed slight chlorosis and stunting effects after application of 2.0 L BAS 736 00 F/ha. Oilseed rape also indicated slight deformations. Plant length was not influenced after application of 2.0 L BAS 736 00 F/ha for all tested plant species. No influence of BAS 736 00 F applied at a rate of 2.0 L/ha on plant dry biomass was observed for all tested plant species, except for carrot and tomato. Carrot showed statistically significant effects on biomass reduction with 6% and tomato with 5% after application of BAS 736 00 F.

**Based on the results of this study, conducted under greenhouse conditions, it can be concluded that BAS 736 00 F applied at BBCH 12 - 14 with a rate of 2.0 L/ha did not cause effects to plant survival and plant length for all tested plant species. Slight phytotoxic effects could be detected for oilseed rape and soybean. No influence of BAS 736 00 F applied at a rate of 2.0 L/ha on plant dry biomass was observed for all tested plant species, except for carrot and tomato with slight but significant reduced biomass of 6% and 5%, respectively. For all tested species,** **the ER50 was estimated to be > 2.0 L BAS 736 00 F/ha.**

**I. MATERIAL AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Dicotyledonous species: carrot (*Daucus carota L*.); lettuce (*Lactuca sativa L*.); cabbage (*Brassica oleracea L. var. capitata L. f. alba*); oilseed rape (*Brassica napus L. ssp. napus*); tomato (*Solanum lycopersicum L*.); soybean (*Glycine max L*.).

Monocotyledonous species: onion (*Allium cepa L.*); ryegrass (*Lolium multiflorum L*.); wheat (*Triticum aestivum L*.); corn (*Zea mays L*.).

Test design: Greenhouse study, limit test; 1 test item rate and 1 tap water control; 5 replicates per treatment, 1 to 3 pots per replicate depending on the species and 2 plants per pot for soybean, tomato and corn, 3 plants per pot for lettuce, oilseed rape and cabbage and 6 plants per pot for carrot, onion, ryegrass and wheat; test item applied post emergence at BBCH 12 - 14 using a laboratory spray chamber at a water volume of 272 L/ha. Assessments for plant damage (phytotoxicity) and plant survival were done 7, 14 and 21 DAT. Single plant length was measured at 21 DAT. Shoot dry weight was determined 21 DAT.

Endpoints: Phytotoxicity (NOER), Plant weight and height (NOER, ER50).

Test rates: Control (tap water), 2.0 L BAS 736 00 F/ha.

Test conditions: Greenhouse conditions, daily average temperature: 21.4 °C – 30.9 °C; daily mean relative humidity: 49.2% - 66.5%; photoperiod: day length ≥ 16 h; additional light when outdoor illumination was < 300 µmol.

Analytics: Analytical verification of the test item was conducted according to BASF method L0361/01 using LC-MS/MS.

Statistics: Descriptive statistics, Student t-test (one-sided smaller, α = 0.05) for metric data, NOER for phytotoxicity was estimated. Phytotoxicity values < 10% were considered as insignificant.

**C. Description of the analytical procedures**

Concentrations of BAS 700 F (contained in BAS 736 00 F) in application solution were determined according to the analytical method L0361/01. After defrosting and subsequent homogenization, the aqueous application solutions were diluted in two steps by a total factor of 100,000 using acetonitrile/water (2/8, v/v) + 0.1% formic acid as solvent. The determination was performed by LC-MS/MS. The limit of quantification (LOQ) achieved was of 0.10 μg/L and the limit of detection (LOD) was set to 0.01 g/L. Due to the high dilution factor of the analyzed solutions, no relevant matrix effects in the LC-MS/MS determination of BAS 700 F were expected. Details on measured fortification samples and obtained procedural recoveries for fluxapyroxad are given in the table below.

**Table A 38: Procedural recoveries for BAS 700 F**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Analyte** | **Fortification level (g/L)** | **n** | **Recoveries (%)** | **Mean recovery (%)** | **RSD (%)** |
| BAS 700 F  Lower fortification level | 0.141 | 1 | 108 | 105 | 2.36 |
| 0.139 | 1 | 108 |
| 0.139 | 1 | 103 |
| 0.135 | 1 | 103 |
| 0.135 | 1 | 105 |
| BAS 700 F  Higher fortification level | 0.273 | 1 | 103 | 103 | 1.74 |
| 0.276 | 1 | 101 |
| 0.287 | 1 | 105 |
| 0.274 | 1 | 101 |
| 0.281 | 1 | 104 |

**II. RESULTS AND DISCUSSION**

After exposure, no plant mortality was observed for all tested plant species following the application of 2.0 L BAS 736 00 F/ha at BBCH stage 12-14. No symptoms of phytotoxicity were observed for all plant species, except for oilseed rape and soybean. Oilseed rape und soybean showed slight chlorosis and stunting effects after application of 2.0 L BAS 736 00 F/ha. Oilseed rape also indicated slight deformations. Plant length was not influenced after application of 2.0 L BAS 736 00 F/ha for all tested plant species. No influence of BAS 736 00 F applied at a rate of 2.0 L/ha on plant dry biomass was observed for all tested plant species, except for carrot and tomato. Carrot showed statistically significant effects on biomass reduction with 6% and tomato with 5% after application of BAS 736 00 F (Student t-test, one-sided smaller α=0.05). The results are summarized in Table A 39 and Table A 40

**Table A 39: Effect of BAS 736 00 F on survival, phytotoxicity, plant length and plant dry weight**

**21 DAT**

| **BAS 736 00 F [L/ha]** | **Carrot** | **Lettuce** | **Cabbage** | **Oilseed**  **rape** | **Tomato** | **Soybean** | **Onion** | **Rye-grass** | **Wheat** | **Corn** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Plant survival [%]** | | | | | | | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
|  | **Phytotoxic damages [%]** | | | | | | | | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.0 | 0 | 0 | 4 C | 18 C, 18D,11 S, | 0 | 10 C, 10 S, | 0 | 0 | 0 | 0 |
|  | **Plant height [% of control]** | | | | | | | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2.0 | 98.1 | 102.1 | 105.2 | 102.3 | 100.3 | 101.8 | 101.1 | 101.1 | 95.1 | 97.1 |
|  | **Plant weight [% of control]** | | | | | | | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2.0 | 94.0\* | 103.4 | 97.3 | 101.5 | 95.4\* | 100.8 | 106.5 | 97.9 | 93.4 | 94.2 |

Phytotoxicity symptoms: Chlorosis (C), Stunting (S), Deformation (D)

\* Statistically significantly different to the control (Student t-test, one-sided smaller α=0.05).

**Table A 40: NOER and ER50 of BAS 736 00 F for non-target plants 21 DAT**

| **Species** | **Carrot** | **Lettuce** | **Cabbage** | **Oilseed**  **rape** | **Tomato** | **Soybean** | **Onion** | **Rye-grass** | **Wheat** | **Corn** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Plant survival [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
| **ER50** | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 |
|  | **Phytotoxic damages\* [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | < 2.0 | ≥ 2.0 | < 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
|  | **Plant height (shoots above ground) [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
| **ER50** | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 |
|  | **Plant weight (shoots above ground) [L/ha]** | | | | | | | | | |
| **NOER** | < 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | < 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
| **ER50** | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 |

\* Estimated from assessment data.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 227** | **Obtained in this study** |
| Seedling emergence is at least 70% | yes (83 – 100%) |
| In the controls: |  |
| The plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species | yes (0%) |
| Mean plant survival at least 90% for the duration of the study | yes (100%) |
| Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source | yes |

All validity criteria were met.

**III. CONCLUSION**

**Based on the results of this study, conducted under greenhouse conditions, it can be concluded that BAS 736 00 F applied at BBCH 12 - 14 with a rate of 2.0 L/ha did not cause effects to plant survival and plant length for all tested plant species. Slight phytotoxic effects could be detected for oilseed rape and soybean. No influence of BAS 736 00 F applied at a rate of 2.0 L/ha on plant dry biomass was observed for all tested plant species, except for carrot and tomato with slight but significant reduced biomass of 6% and 5%, respectively. For all tested species, the ER50 was estimated to be > 2.0 L BAS 736 00 F/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. |

|  |  |
| --- | --- |
| Reference: | CP 10.6.2/2 |
| Report | Effect of BAS 736 00 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions,  Maleck, A., 2019  report No 849656, AC/BASF/19/19  2019/1061110  Authority registration No |
| Guideline(s): | EPA 850.4100 - Seedling Emergence and Seedling Growth (2012), OECD 208 |
| Deviations: | No |
| GLP: | yes  (certified by Land Brandenburg Ministerium der Justiz und fuer Europa und fuer Verbraucherschutz, Potsdam, Germany), | |
| Acceptability: | Yes |
| Duplication  (if vertebrate study) | No |

**Executive Summary**

In a seedling emergence test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean and tomato) and four species of monocotyledonous plants (onion, ryegrass, wheat and corn) were exposed to BAS 736 00 F to evaluate the phytotoxic potential. BAS 736 00 F was applied pre-emergence at a limit rate of 2.0 L BAS 736 00 F/ha. After application, the seeds were cultivated for 21 days under greenhouse conditions (28 days for carrot and onion). Assessment of seedling emergence, plant survival and plant damage (phytotoxicity) were done 7, 14 and 21 days after treatment (DAT; 14, 21 and 28 DAT for carrot and onion). Assessments of shoot dry weight and single plant length was determined 21 DAT (28 DAT for onion and carrot).

After exposure, none of the tested plant species was affected concerning seedling emergence and plant survival by the application of 2.0 L BAS 736 00 F/ha. Furthermore, all tested species showed no phytotoxic symptoms after use of 2.0 L BAS 736 00 F/ha pre emergence. No reduction of plant length and biomass was observed for all tested plant species following the application of 2.0 L BAS 736 00 F/ha.

**Based on the results of this study, conducted under greenhouse conditions, it can be concluded that BAS 736 00 F at a rate of 2.0 L product/ha did not cause effects to the seedling emergence, plant survival, plant length and plant biomass of the tested plant species. For all tested species, the ER50 was estimated to be > 2.0 L BAS 736 00 F/ha.**

**I. MATERIALS AND METHODS**

**A. MATERIALS**

Test item: BAS 736 00 F; batch no. FD-190220-0002; content of a.s.: azoxystrobin (BAS 9164 F, Reg. No. 300 254): 73.9 g/L analyzed (75.0 g/L nominal), fluxapyroxad (BAS 700 F, Reg. No. 5 094 351): 49.4 g/L analyzed (50.0 g/L nominal); density: 1.078 g/cm³.

**B. STUDY DESIGN**

Test species: Dicotyledonous species: Carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* L. *var. capitata*), oilseed rape (*Brassica napus* L*.* spp. *napus*), tomato (*Solanum lycopersicum L.*), soybean (*Glycine max* L.).

Monocotyledonous species: onion (*Allium cepa* L.), ryegrass (*Lolium multiflorume* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays L.*).

Test design: Greenhouse study, limit test; 1 test item rate and 1 tap water control; 4 replicates per treatment, 1 to 2 pots per replicate depending on the species and 5 seeds per pot for soybean, tomato, corn, lettuce, oilseed rape and cabbage and 10 plants per pot for carrot, onion, ryegrass and wheat; test item applied pre-emergence using a laboratory spray chamber at a water volume of 265 L/ha. Assessments for seedling emergence, plant survival and plant damage (phytotoxicity) were done 7, 14 and 21 DAT (carrot and onion 14, 21 and 28 DAT). Shoot dry weight and single plant length was determined 21 DAT (28 DAT for onion and carrot).

Endpoints: Phytotoxicity (NOER), Plant emergence, weight and height (NOER, ER50).

Test rates: Control (tap water), 2.0 L BAS 736 00 F/ha.

Test conditions: Greenhouse conditions, daily average temperature: 23.7 °C – 29.0 °C; daily mean relative humidity: 52.0% - 68.6%; photoperiod: day length ≥ 16 h; additional light when outdoor illumination was < 300 µmol.

Analytics: Analytical verification of the test item was conducted according to BASF method L0361/01 using LC-MS/MS.

Statistics: Descriptive statistics, Student t-test (one-sided smaller, α = 0.05) for metric data, Fisher’s Exact test (one-sided greater, α = 0.05) for quantal data, NOER for phytotoxicity was estimated. Phytotoxicity values < 10% were considered as insignificant.

**C. Description of the analytical procedures**

Concentrations of BAS 700 F (contained in BAS 736 00 F) in application solution were determined according to the analytical method L0361/01. After defrosting and subsequent homogenization, the aqueous application solutions were diluted in two steps by a total factor of 100,000 using acetonitrile/water (2/8, v/v) + 0.1% formic acid as solvent. The determination was performed by LC-MS/MS. The limit of quantification (LOQ) achieved was of 0.10 μg/L and the limit of detection (LOD) was set to 0.01 g/L. Due to the high dilution factor of the analyzed solutions, no relevant matrix effects in the LC-MS/MS determination of BAS 700 F were expected. Details on measured fortification samples and obtained procedural recoveries for fluxapyroxad are given in the table below.

**Table A 41: Procedural recoveries for BAS 700 F**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Analyte** | **Fortification level (g/L)** | **n** | **Recoveries (%)** | **Mean recovery (%)** | **RSD (%)** |
| BAS 700 F  Lower fortification level | 0.284 | 1 | 105 | 102 | 1.97 |
| 0.276 | 1 | 100 |
| 0.285 | 1 | 101 |
| 0.282 | 1 | 102 |
| 0.286 | 1 | 101 |
| BAS 700 F  Higher fortification level | 0.469 | 1 | 102 | 103 | 1.33 |
| 0.465 | 1 | 102 |
| 0.467 | 1 | 103 |
| 0.474 | 1 | 105 |
| 0.464 | 1 | 128 |

**II. RESULTS AND DISCUSSION**

After exposure, none of the tested plant species was affected concerning seedling emergence and plant survival by the application of 2.0 L BAS 736 00 F/ha. Furthermore, all tested species showed no phytotoxic symptoms after use of 2.0 L BAS 736 00 F/ha pre emergence. No reduction of plant length and biomass was observed for all tested plant species following the application of 2.0 L BAS 736 00 F/ha. The results are summarized in Table A 42 and Table A 43.

**Table A 42: Effect of BAS 736 00 F on seedling emergence, phytotoxicity, plant survival, plant**

**length and plant dry weight 21 DAT**

| **BAS 736 00 F [L/ha]** | **Carrot\*** | **Lettuce** | **Cabbage** | **Oilseed**  **rape** | **Tomato** | **Soybean** | **Onion\*** | **Rye-grass** | **Wheat** | **Corn** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Plant emergence [%]** | | | | | | | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2.0 | 115 | 100 | 109 | 100 | 94 | 103 | 106 | 103 | 95 | 87 |
|  | **Plant survival [%]** | | | | | | | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
|  | **Phytotoxic damages [%]** | | | | | | | | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|  | **Plant height [% of control]** | | | | | | | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2.0 | 99.8 | 101.5 | 100.6 | 99.5 | 96.3 | 97.6 | 100.6 | 97.6 | 99.3 | 101.9 |
|  | **Plant weight [% of control]** | | | | | | | | | |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2.0 | 111.7 | 102.7 | 110.4 | 102.0 | 90.4 | 101.3 | 102.5 | 101.4 | 101.0 | 96.0 |

\* Carrot and onion 28 DAT.

**Table A 43: NOER and ER50 of** **BAS 736 00 F for non-target plants 21 DAT**

| **Species** | **Carrot\*** | **Lettuce** | **Cabbage** | **Oilseed**  **rape** | **Tomato** | **Soybean** | **Onion\*** | **Rye-grass** | **Wheat** | **Corn** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Seedling emergence [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
| **ER50** | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 |
|  | **Plant survival [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
| **ER50** | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 |
|  | **Phytotoxic damages\*\* [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
|  | **Plant height (shoots above ground) [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
| **ER50** | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 |
|  | **Plant weight (shoots above ground) [L/ha]** | | | | | | | | | |
| **NOER** | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 | ≥ 2.0 |
| **ER50** | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 | > 2.0 |

\* Carrot and onion 28 DAT.

\*\* Estimated from assessment data.

Validity criteria:

|  |  |
| --- | --- |
| **Validity criteria according to OECD 208** | **Obtained in this study** |
| Seedling emergence is at least 70% in the control | yes (80% to 100%) |
| Seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) in the control and control plants exhibit only normal variation in growth and morphology for that particular species | yes (0%) |
| Mean survival of emerged control seedlings at least 90% for the duration of the study | yes (100%) |
| Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source | yes |

All validity criteria were met.

**III. CONCLUSION**

**Based on the results of this study, conducted under greenhouse conditions, it can be concluded that BAS 736 00 F at a rate of 2.0 L product/ha did not cause effects to the seedling emergence, plant survival, plant length and plant biomass of the tested plant species. For all tested species, the ER50 was estimated to be > 2.0 L BAS 736 00 F/ha.**

* + 1. KCP 10.6.3 Extended laboratory studies on non-target plants

As BAS 736 00 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 736 00 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)

Not relevant.

* 1. KCP 10.8 Monitoring data

Not relevant.