European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

BAS 750F
(Mefentrifluconazole)

Volume 3 – B.9 (PPP) – BAS 750 01 F

Rapporteur Member State: United Kingdom
Co-Rapporteur Member State: France & Austria

Version History

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

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

BAS 750 01 F is the representative formulation for the approval of the new fungicidal active substance BAS 750 F. BAS 750 01 F is an EC (emulsifiable concentrate) formulation, containing 100 g BAS 750 F/L.

The risk assessment for birds and mammals for BAS 750 F is conducted according to the latest guidance document by EFSA (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.
According to Section 4 of 283/2013 and Section 5 of 284/2013 there is a need to ensure that there are appropriate methods of analysis, i.e. in Section 4.1.2 of 283/2013, it is stated that "methods shall be submitted, with a full description, for the determination of non-isotope-labelled residues in all areas of the dossier, as set out in detail in the following points: (f) in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies;"

The RMS has considered the method of analysis used for the ecotoxicological studies and the associated assessment is presented in III CA B.5.1.2.6 and IIICP B.5.1.2. In assessing these methods of analysis, several were deemed to comply with the criteria outlined in SANCO/3029/99 rev.4 and these are considered to be acceptable and hence can be used, without further consideration, for risk assessment purposes. In several instances, the following deficiencies were identified:

- *The suitability of matrix matched standards for calibration purposes has not been addressed*
- *Only 3 instead of 5 replicates have been used to determine recoveries and repeatability at each fortification level*
- *The linear range has not been demonstrated to be appropriate to the concentrations of the analyte in relevant analytical matrices*
- *In one method deficiencies in accuracy which may lead to an underestimation of the toxicity of BAS 750 F*

Presented below is a consideration of each of these deficiencies:

Linearity
SANCO/3029/99 rev.4 states that the analytical calibration should extend over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions ± at least 20%. In the validation data for some of the methods, the range does not cover the concentration of the analyte in the study. Whilst important, the RMS is of the view that this does not fundamentally undermine the validity of the studies concerned as when the method is used the samples can be diluted to bring them into the range of the calibration curve.

Repeatability
SANCO/3029/99 rev.4 states that five determinations should be made at each fortification level to determine relative standard deviation (RSD) of repeatability. In the validation data for some of the methods, only three determinations were made as opposed to five. The RMS is of the view that as the RSD in each case is below the acceptable limit of 20% given in the guidance that this provides sufficient confidence in the repeatability of the method. In the majority of the validation data at least two fortification levels were used, giving a total of at least six determinations, and the RSD for the six fortifications was also less than 20%, which supports the acceptability of the recovery.

Matrix matching
SANCO/3029/99 rev.4 states that when determining linearity the possible effects of sample components, e.g. co-extractives, on chromatographic transmission or detection system response must be addressed, and that where appropriate, detection system calibration should be generated using standard solutions in a matrix similar to that of the samples to be analysed. In the validation data for some of the methods to support ecotoxicology studies, linearity was determined using calibration (solvent) standards, rather than matrix matched standards. Where matrix matched standards are not used, this could result in suppression or enhancement of recovery, i.e. an over or under prediction of the actual residue level. In some circumstances other data may provide mitigation for the absence of matrix matched standards for a pre-registration method, however this depends on the nature of the method. The impact of the absence of matrix matched standards on the suitability of the methods of analysis is therefore considered on a case by case basis, taking into account the impact of inaccuracies in the determined residue levels on the risk assessment.

Following consideration of additional data supplied by the applicant, it was considered that the methods of analysis used within ecotoxicological studies provided for BAS 750 F were sufficiently validated for the purposes of the regulatory process (III CP B.5.1.2).

B.9.1.1. Effects on birds

The avian toxicity studies with BAS 750 F relevant for the risk assessment for birds according to EFSA/2009/1438 are summarised in Table B.9.1.1-1.

Table B.9.1.1-1 Toxicity endpoints and effect values for the risk assessment for birds for BAS 750 F

Species	Substance	Exposure System	Results	Reference
Colinus virginianus	BAS 750 F	Oral, 1 d Acute	LD_{50} = 816 mg/kg bw	III CA B.9.1.1.1/1 [redacted] 2014a
Anas platyrhynchos	BAS 750 F	Oral, 1 d Acute	LD_{50} > 2000 mg/kg bw	III CA B.9.1.1.1/2 [redacted] 2014b
Serinus canaria	BAS 750 F	Oral, 1 d Acute	LD_{50} > 2860 mg/kg bw	III CA B.9.1.1.1/3 [redacted]. 2015a
Colinus virginianus	BAS 750 F	Dietary Reproductive toxicity	NOEL = 25.3 mg/kg bw/d	III CA B.9.1.1.3/1 [redacted] 2014a
Anas platyrhynchos	BAS 750 F	Dietary Reproductive toxicity	NOEL = 80.5 mg/kg bw/d	III CA B.9.1.1.3/2 [redacted] 2015a
Endpoint used for acute assessment	BAS 750 F	Oral, 1 d	**LD_{50} (extrapolated, geomean) = 2065.5 mg/kg bw**	Geomean of quail, canary, and extrapolated mallard LD_{50}s
Endpoint used for reproductive assessment	BAS 750 F	**Dietary Reproductive toxicity – Tier 1**	**NOEL = 25.3 mg/kg bw/d**	III CA B.9.1.1.3/1 [redacted] 2014a

Justification for endpoints used in risk assessment

Acute – Three acute oral acceptable toxicity studies are available for birds. However, according to regulation 384/2013, only one is required. It is assumed that the additional studies were performed for other regulatory authorities. For acute toxicity, the critical endpoint is an LD_{50} for 816 mg/kg bw for *Colinus virginianus*. Because no mortality or sublethal effects occurred in the mallard acute study (III CA B.9.1.1.1/2), the endpoint (LD_{50} > 2000 mg/kg bw) was extrapolated to LD_{50} = 3776 mg/kg bw (see 2.1.2 of EFSA Guidance (2009)). The geomean of the quail, canary, and extrapolated mallard endpoints is LD_{50} = 2065.5 mg/kg bw. According to EFSA (2009) the geometric mean should be used for the acute assessment, except when the endpoint for the most sensitive species is more than a factor of 10 below the geometric mean of all the tested species. As this is not the case, the geometric mean of the acute studies is suitable as a refinement for use in the risk assessments.

Reproductive – As the lowest chronic endpoint, NOEL = 25.3 mg/kg bw/d from the quail reproduction study is used in the chronic risk assessment.

According to Commission Regulation (EU) 283/2013 and 284/2013 estimates of EC_x (e.g. EC_{10}, EC_{20}) together with the NOEL for chronic studies are required. No EC_x values have been produced for the reproduction studies; the Applicant has argued that "avian reproduction studies were designed for deriving a NOEL value. Since only three widely spaced dietary concentrations were tested, this study design is not suitable for calculating EC_x values. Additionally, the risk assessment and trigger values in the bird and mammal risk assessment guidance document (EFSA/2009/1438) are based on the use of NOEL values, so the NOEL is the appropriate value to use in the avian risk assessment. Furthermore, according to EFSA (2015)[1] "the test guideline has serious limitation for the derivation of reliable EC_{10} estimations", hence the RMS is an agreement with the Applicant.

Relevant exposure scenarios

The intended representative use pattern of the formulation BAS 750 01 F is given in the Table below.

Table B.9.1.1-2: Proposed use pattern of BAS 750 F

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				BAS 750 F [kg a.s.//ha]	BAS 750 01 F [kg/ha]
Cereals	30-69	2	14	0.150	1.5

[1] EFSA (European Food Safety Authority), 2015. 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.

B.9.1.2. Effects on terrestrial vertebrates other than birds

The mammalian toxicity studies with BAS 750 F relevant for the risk assessment for wild mammals are summarised in Table B.9.1.2-1.

Table B.9.1.2-1: Toxicity endpoints and effect values for the risk assessment for mammals for BAS 750 F

Species	Substance	Exposure System	Results	Reference
Rat	BAS 750 F	Oral, 1 d Acute	LD_{50} > 2000 mg as/kg bw	[redacted], 2013c (CA 5.2.1/1)
Rat	BAS 750 01 F	Oral, 1 d Acute	LD_{50} > 2000 mg formulation/kg bw	[redacted], 2015a (CP 7.1.1/1)
Rat	BAS 750 F	Dietary Reproductive toxicity Two-generation study	$NOEL_{Reproduction}$ = 200 mg a.s./kg bw/d $NOEL_{Parents}$ = 25 mg a.s./kg bw/d $NOEL_{Offspring}$ = 75 mg a.s./kg bw/d	[redacted] 2015c (CA 5.6.1/1)
Rat	BAS 750 F	Oral Developmental toxicity	$NOEL_{Maternal}$ = 150 mg a.s./kg bw/d $NOEL_{Developmental}$ = 400 mg a.s./kg bw/d	[redacted] 2015a (CA 5.6.2/1)
Rabbit	BAS 750 F	Oral Developmental toxicity	$NOEL_{Maternal}$ = 25 mg a.s./kg bw/d $NOEL_{Developmental}$ = 25 mg a.s./kg bw/d	[redacted] 2015b (CA 5.6.2/2)
Endpoint used for acute AI assessment	**BAS 750 F**	**Oral, 1 d Acute**	**LD_{50} > 2000 mg as/kg bw**	[redacted] 2013c (CA 5.2.1/1)
Endpoint used for acute formulation assessment	**BAS 750 01 F**	**Oral, 1 d Acute**	**LD_{50} > 2000 mg formulation/kg bw**	[redacted], 2015a (CP 7.1.1/1)
Endpoint used for reproductive assessment	**BAS 750 F**	**Dietary Reproductive toxicity – Tier I**	**NOEL = 25 mg as/kg bw/d**	[redacted] 2015c (CA 5.6.1/1)

Justification for endpoints used in risk assessment

Acute – As the only acute mammal endpoint, LD_{50} > 2000 mg/kg bw from the rat study is used in the acute risk assessment.

Reproductive – As the lowest chronic endpoint, NOEL = 25 mg/kg bw/d from the dietary reproductive toxicity two-generation study rabbit developmental study is used in the chronic risk assessment. For the critical endpoint, $NOAEL_{parents}$, at the next concentration, 75mg/kg bw/d, increased

cholesterol in males, liver weight in males and females, and increased alkaline phosphatase concentrations in males and females was reported.

According to Commission Regulation (EU) 283/2013 and 284/2013 estimates of EC_x (e.g. EC_{10}, EC_{20}) together with the NOEL for chronic studies are required. No EC_x values have been produced for the reproduction studies; the Applicant has argued that "mammalian reproduction studies were designed for deriving a NOEL value. Since only three widely spaced dietary concentrations were tested, this study design is not suitable for calculating EC_x values. Additionally, the risk assessment and trigger values in the bird and mammal risk assessment guidance document (EFSA/2009/1438) are based on the use of NOEL values, so the NOEL is the appropriate value to use in the avian risk assessment. Furthermore, according to EFSA (2015)[2] "the test guideline has serious limitation for the derivation of reliable EC_{10} estimations"; hence the RMS is in agreement with the Applicant.

Relevant exposure scenarios

The intended representative use patterns of the formulation BAS 750 01 F is given in the Table below.

Table B.9.1.2-2: Proposed use pattern

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				BAS 750 F [kg a.s.//ha]	BAS 750 01 F [kg/ha]
Cereals	30-69	2	14	0.150	1.5

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

Birds

Acute toxicity to birds

Table B.9.1.1-3: Acute dietary risk assessment for birds in cereals-results

	Crop	Application rate (Kg a.s./ha)	Number of applications	Application Interval	DT_{50}	LD_{50}	
	Cereals	0.15	2	14	10.0	816	
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	1.2	28.58	28.55	

In conclusion, under the assumptions of the screening step, all TER_A values for BAS 750 01 F exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 750 01 F according to the proposed use pattern.

[2] EFSA (European Food Safety Authority), 2015. 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.

Reproductive toxicity to birds

Table B.9.1.1-3: Reproductive risk assessment for birds in cereals-results

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.15	2	14	10	25.3	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 omnivorous bird	64.8	9.72	1.4	7.21	**3.5**	

First Tier Risk Assessment:					
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**	**Shortcut value**	**TER**	**No refinement required**
	Cereals BBCH 30 - 39	Small omnivorous bird “lark”	5.4	42.1	
	Cereals BBCH ≥ 40	Small omnivorous bird “lark”	3.3	68.9	

In conclusion, for the tier 1 risk assessment, all TER_{LT} values for BAS 750 01 F exceed the trigger value set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low long-term/ reproductive risk for birds from the use of BAS 750 01 F according to the proposed use pattern.

Formulation toxicity

According to Commission regulation (EU) 284/2013, an avian acute test with the formulation is required if toxicity cannot be predicted on the basis of the data for the active substance, or where results from mammalian testing give evidence of higher toxicity of the plant protection product compared to the active substance.

For BAS 750 01 F, the acute oral study in rats resulted in LD_{50} > 200 mg a.s./kg bw (see chapter III CP B.6.1.1). Given that this is a greater than value as part of a limit test (>2000 mg f.p./kg bw) and all clinical signs were resolved by the end of the day of dose administration, despite being a smaller value when expressed in terms of active substance (> 200 mg a.s./kg bw for the formulated product compared to > 2000 mg a.s./kg bw for the active substance) there is no indication that the formulated product is any more toxic than the active substance. Consequently, no acute oral tests with birds on the product are considered necessary and toxicity can reliably be predicted on the basis of the data for BAS 750 F.

BAS 750 01 F is a solo formulation with BAS 750 F being the only active substance. Therefore, an assessment of mixture toxicity is not applicable.

Food chain from earthworm to earthworm-eating birds

The risk assessment for earthworm-eating birds will be based on the worst case PEC_{soil} (max) derived from the environmental fate section (see section III CP B.8.1.3). The calculations and the resulting TER_{LT} values are summarised in Table B.9.1.1.2-2.

Table B.9.1.1.2-2: Risk assessment for the active substance BAS 750 F concerning earthworm-eating birds (Tier 1) [1)]

Parameter	BAS 750 F	Reference
PEC_{soil} (max) [mg/kg soil] [2)]	0.080	Sections III CP B.8.2.1.1, Table III CP 8.2.1.1.1
K_{OW}	2350[7)]	III CA B.2.7/01
K_{oc} (geometric mean)	3455.6[8)]	III CA B.8.5, Table 8.3.5
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [3)]	0.420	--
PEC_{worm} [4)]	0.0336	--
Daily dose [mg/kg b.w./day] [5)]	0.0353	--
NOEL [mg/kg b.w./day]	25.3	See above
TER_{LT} [6)]	**716.8**	--

1 According to EFSA/2009/1438
2 Worst case PEC_{soil} (max) value calculated for twofold application of 150 g a.s./ha to cereals. For details see section Table 8.2.1.1.1
3 Bioconcentration factor (BCF) = $(0.84 + 0.012 * K_{OW}) / f_{oc} * K_{oc}$
4 $PEC_{worm} = PEC_{soil} * BCF$
5 Daily dose = $1.05 * PEC_{worm}$
6 TER_{LT} = NOEL / Daily dose
7 III CA B.2.7 lists $\log K_{OW} = 3.4$. For K_{OW} value, see Section B.2.7/01, Wilbrand S. 2013c
8 Geometric mean K_{oc} of n=8 soils used

Food chain from fish to fish eating birds

The risk assessment for fish-eating birds is based on the PEC_{SW} (max. Step 1) derived from the environmental fate section (see section III CP B.8.2.5). The calculations and the resulting TER_{LT} values are presented in Table B.9.1.1.2-3.

Table B.9.1.1.2-3: Risk assessment for the active substance BAS 750 F concerning fish-eating birds (tier 1) [1)]

Parameter	BAS 750 F	Reference
$PEC_{SW,}$ (max. Step 1) [mg/L] [2)]	0.020592	III CP B.8.5, Tables 8.5-7 and 8.5-8
BCF fish (max. worst case)	385	B.9.2.2/7, [redacted] 2015c
PEC_{fish} [mg/kg] [3)]	7.9279	--
Daily dose [mg/kg b.w./d] [4)]	1.261	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	25.3	See above
TER_{LT} [5)]	20.07	--

1 According to EFSA/2009/1438

2 PEC_{SW} (max, Step 1) resulting from FOCUS Step 1 150g a.s./ha application on winter or spring cereals
3 $PEC_{fish} = PEC_{SW,}$ (max, Step 1) x BCF
4 Daily dose = 0.159 x PEC_{fish}
5 TER_{LT} = NO(A)EL / Daily dose.

In conclusion, according to the tier 1 risk assessment for earthworm- and fish-eating birds, the TER values for BAS 750 F are above the trigger set by Commission regulation (EU) 546/2011, i.e. ≥ 5 for reproductive exposure, indicating a low risk for birds from the use of BAS 750 01 F according to the proposed use pattern.

Risk for birds through drinking water

Of the two drinking water risk assessment scenarios for birds in EFSA/2009/1438, *i.e.* the leaf and the puddle scenario, the leaf scenario is not relevant for use in cereals. Consequently, the 'puddle scenario' will be considered for the application of BAS 750 01 F in cereals.

Puddle scenario
According to EFSA/2009/1438, no specific calculations of exposure and TER values 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 (K_{oc} < 500) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500). The ratio for acute and reproductive endpoints for BAS 750 F (0.142 and 11.56, respectively) do not exceed the threshold value of 3000 as given by EFSA/2009/1438 for more sorptive substances (K_{oc} ≥ 500), thus no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary (Table B.9.1.1.2-4).

Table B.9.1.1.2-4: Screening step for drinking water risk assessment in cereals-ratio of effective application rate to relevant endpoint for birds

Parameter	BAS 750 F	Reference
K_{oc} (geometric mean)	3455.6 [6]	III CA B.8.5, Table 8.3.5
DT_{50} (soil) [d]	200	III CA B.8, Table 8.2
Number of applications	2	See use pattern
Interval [d]	14	See use pattern
MAF_m [1]	1.95	--
Max use rate [g/ha]	150	See use pattern
AR_{eff} [g/ha] [2]	292.5	--
LD_{50} [mg/kg b.w.]	2065.5	See above
Ratio (acute) [3]	0.142	--
NO(A)EL [mg/kg b.w./d]	25.3	See above
Ratio (repro) [3]	11.56	--
Trigger [4]	3000	--
Drinking water assessment required [Yes/No] [5]	No	--

1 $MAF_m = (1-e^{-nki}) / (1-e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d].
2 AR_{eff} = Application rate (g/ha) x MAF_{mean}
3 Ratio of AR_{eff} and relevant toxicity endpoint
4 Trigger according to EFSA/2009/1438
5 Drinking water risk assessment is not necessary when trigger value is not exceeded.
6 Geometric mean K_{oc} of n=8 soils used

In conclusion, a quantitative drinking water risk assessment for the puddle scenario is not triggered for birds, indicating a low risk for the intended use of BAS 750 01 F in cereals.

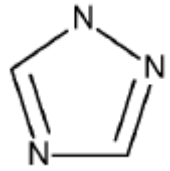
Metabolite assessment

In environmental metabolism studies (see chapters III CA B.6.1 and III CA B.6.2) conducted for BAS 750 F, metabolites M750F005, M750F006, M750F007, M750F008 approached or exceeded 10% TAR in water in the aqueous photolysis study (III CA B.8.2.1.2, Zhixing Y., 2015a). Metabolite M750F001 exceeded 10% TAR in water in the aerobic aquatic metabolism study (III CA B.8.2.2.3/1, Ebert D.,Dalkmann P., 2015 a), while no metabolite approached or exceeded 10% TAR in sediment or soil. According to EFSA/2009/1438, a log $K_{OW} \geq 3$ indicates a potential for bioaccumulation. Since the log K_{OW} values for these metabolites are < 3 (0.41 for M750F003, 1.69 for M750F005, 2.73 for M750F006, 0.9 for M750F007, 1.76 for M750F008 (see Section B.2.14), the risk of secondary poisoning to birds and mammals from consumption of fish and earthworms is considered low. For M750F001, the log K_{OW} is -1 (EFSA Scientific Report (2008), PRAPeR expert meeting 138).

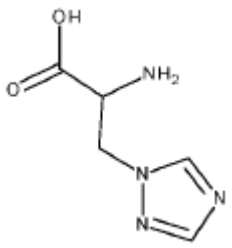
In the plant metabolism studies with BAS 750 F on soybeans, wheat, and grapes (BASF DocIDs 2014/1224012, 2015/1001872, 2015/1073822; see chapter III CA B.7.2), the metabolites M750F029 (triazole alanine) and M750F030 (triazole acetic acid) approached or exceeded 10% TRR in potential food items for birds and mammals (i.e. forage, grains, fruits). In the rotational crop study with BAS 750 F on spinach, wheat, and radishes (III CA B.7.6), the metabolites M750F001 (1,2,4-triazole), M750F029, M750F030, and M750F031 (triazole lactic acid) approached or exceeded 10% TRR on potential food items for birds and mammals (i.e. forage, grains, fruits).

The potential relevance of these metabolites is as follows:

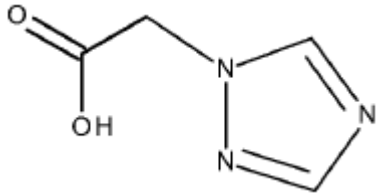
- **Metabolite M750F001 (1,2,4-triazole)** was detected in significant amounts in metabolism studies with hens (max TRR 91.4% in muscle and max concentration 0.409 mg/kg bw (85.2% TRR) in liver, III CA B.7.2.2), lactating goats (max TRR 95.2% in skimmed milk and 87.3% in muscle, and max concentration 0.270 mg/kg bw (68.1% TRR) in kidney, III CA B.7.2.3). This metabolite is therefore covered by the toxicological investigations with the parent compound in vertebrates.



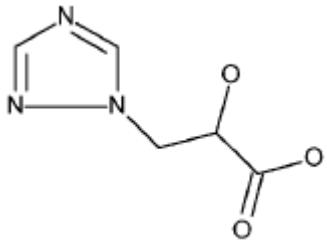
- **Metabolite M750F029 (triazole alanine)** was not detected in animal metabolism studies on BAS 750 F. The toxicity of M750F029 to rats and mice is LD_{50} > 5000 mg/kg bw [1)] and 2 generation reproduction NOEL = 100 mg/kg bw/d [1)] (EFSA Scientific Report (2006), PRAPeR expert meeting 13). Additionally, the toxicity of this metabolite to birds is LC_{50} > 1342 mg/kg diet [1)] for both bobwhite quail and mallard duck (EFSA Scientific Report (2006), PRAPeR expert meeting 13). Based upon the EU agreed endpoints above, there is no evidence that the toxicity of this metabolite is greater than that of the active substance. Therefore the risk posed by the active substance covers this metabolite.



- **Metabolite M750F030 (triazole acetic acid)** was not detected in animal metabolism studies on BAS 750 F. The toxicity of M750F030 to rats is LD_{50} > 5000 mg/kg bw [3)] and 2 generation reproduction NOEL = 100 mg/kg bw/d [1)] (EFSA Scientific Report (2016), EFSA-Q-2016-00457). Additionally, the toxicity of this metabolite to bobwhite quail is LD_{50} > 2000 mg/kg diet [1)] (EFSA Scientific Report (2006), PRAPeR expert meeting 13). Based upon the EU agreed endpoints above, there is no evidence that the toxicity of this metabolite is greater than that of the active substance. Therefore the risk posed by the active substance covers this metabolite.



- **Metabolite M750F031 (triazole lactic acid)** has a very similar structure to that of M750F029, although with a substitution of the amine group adjacent the carboxylic acid with an ethanol group. This results in the loss of the aliphatic amines-acid class group, and due to the structural similarity, the toxicity is expected to be similar to that of M750F029 (EFSA Scientific Report (2016), EFSA-Q-2016-00457), and therefore covered by the risk of the active substance.



Additionally, residues of these four metabolites measured in plant material were far lower than the maximum RUDs of 15.3 mg/kg (102.3 mg/kg x 0.15 kg a.s./ha = 15.3 mg/kg) and 8.13 mg/kg (54.2 mg/kg x 0.15 kg a.s./ha = 8.13 mg/kg) used in acute and chronic risk assessments, respectively, for the parent compound. Maximum measured metabolite concentrations were 0.339 mg M750F001/kg, 2.361 mg M750F029/kg, 0.689 mg M750F030/kg, and 0.319 mg M750F031/kg in wheat grain after application of 300 g a.s./ha to soil (III CA B.7.2.1.2), corresponding to the proposed application rate of 2 x 150 g a.s./ha for the representative formulation.

In summary, no secondary poisoning assessment is necessary for any metabolite of BAS 750 F because for all metabolites approaching or exceeding 10% TAR in environmental metabolism studies, the metabolite log K_{OW} values are < 3. For metabolites approaching or exceeding 10% TRR in potential food items for birds and mammals (i.e. forage, grains, fruits) in plant metabolism studies, the risk assessment for the parent compound covers the risk for birds and mammals from the metabolites.

[1)] Cited studies on the toxicity of M750F029 and M750F030 to birds were previously evaluated at the EU level as reviewed in the draft assessment report for epoxiconazole by the rapporteur member state Germany, Vol. 3, B.9 (March 2006). Cited studies on the toxicity of M750F029 and M750F030 to mammals were also previously evaluated on the EU level. [redacted] (2010) was reviewed in the UK's confirmatory data addendum for triazole derivative metabolites (2015). For details on these studies, consult the stated documents.

Overall conclusion

In conclusion, under the assumptions of the Tier I risk assessment, all TER values for the dietary acute and long-term, and secondary poisoning risk assessments for BAS 750 01 F exceed the trigger value set by Commission regulation (EU) 546/2011 for acceptability of effects, and the ratio of the effective application rate to the relevant toxicity endpoint indicates that a quantitative risk assessment for drinking water exposure is not required. This indicates a low risk for birds from use of BAS 750 01 F according to the proposed use pattern.

Mammals

Relevant exposure scenarios

The intended representative use patterns of the formulation BAS 750 01 F is given in the Table below.

Table B.9.1.2-2: Proposed use pattern

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				BAS 750 F [kg a.s.//ha]	BAS 750 01 F [kg/ha]
Cereals	30-69	2	14	0.150	1.5

Acute oral toxicity to mammals

Table B.9.1.2.1-1: Acute risk assessment for mammals in cereals-results

	Crop	Application rate (Kg a.s./ha)	Number of applications	Application Interval	DT_{50}	LD_{50}	
	Cereals	0.15	2	14	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 herbivorous mammal	118.4	17.76	1.2	21.31	> 93.8	

In conclusion, under the assumptions of the screening step, all TER_A values for BAS 750 01 F exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for mammals from the use of BAS 750 01 F according to the proposed use pattern.

Reproductive risk to mammals

Table B.9.1.2.2-1: Reproductive risk assessment for mammals in cereals-results

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.15	2	14	10	25	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	7.25	1.4	5.38	**4.65**	

First Tier Risk Assessment:					
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**	**Shortcut value**	**TER**	**Trigger**
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	118.2	5
	Cereals BBCH 30-39	Small omnivorous mammal “mouse”	3.9	57.6	
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole”	21.7	10.4	
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse”	2.3	97.7	

In conclusion, under the assumptions of the tier 1 assessment, all TER_{LT} values for BAS 750 01 F exceed the trigger value set by Commission regulation (EU) 546/2011 for acceptability of effects, indicating a low risk for mammals from use of BAS 750 01 F according to the proposed use pattern.

Formulation toxicity

Although the risk posed by the formulation has been concluded as being covered by the active substance, a risk assessment for the formulated product has been included below for illustrative purposes. An acute oral toxicity study for the rat for the formulation BAS 750 01 F resulted in LD_{50} > 2000 mg formulation/kg bw (III CP B.6.1.1). Taking into account the formulation density of 0.993 g/cm^3, this results in a single application rate of 1.4895 kg BAS 750 01 F/ha in cereals.

Table B.9.1.2.2-2: Acute risk assessment of the formulation for mammals in cereals-results

	Crop	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	**Time weighted average (TWA)**
	Cereals	1.4895	2	14	10.0	> 2000	0.53
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 herbivorous mammal	118.4	176.36	1.2	211.63	**>9.45**	

First Tier Risk Assessment:					
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**	**Shortcut value**	**TER**	**Trigger**
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	5.4	>207.2	10
	Cereals BBCH 30-39	Small omnivorous mammal “mouse”	8.6	>130.1	
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole”	40.9	>27.4	
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse”	5.2	>215.2	

In conclusion, under the assumptions of the tier 1 assessment, all TER_A values for the formulation BAS 750 01 F exceed the trigger of 10 set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for mammals from the use of the formulation according to the proposed use pattern.

Effects of secondary poisoning

The log K_{OW} of the active substance BAS 750 F was determined to be 3.4 at pH 7 (III CA B.2.7/01, Wilbrand S. 2013c). Hence, an assessment of the potential risk of secondary poisoning according to EFSA/2009/1438 is triggered.

Food chain from earthworm to earthworm-eating mammals

The risk assessment for earthworm-eating mammals is based on the worst case PEC_{soil} (max) derived from the environmental fate section (see section III CP B.8.1.3). The calculations and the resulting TER_{LT} values are presented in Table B.9.1.2.2-3.

Table B.9.1.2.2-3: Risk assessment for the active substance BAS 750 F concerning earthworm-eating mammals (tier 1) – dry soil approach [1)]

Parameter	BAS 750 F	Reference
PEC_{soil} (max) [mg/kg soil] [2)]	0.080	III CP B.8.2.1.1, Table 8.2.1.1.1
K_{OW}	2350[7)]	III CA B.2.7/01
K_{oc}	3455.6[8)]	III CA B.8.5, Table 8.3.5
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [3)]	0.420	--
PEC_{worm} [mg/kg] [4)]	0.0336	--
Daily dose [mg/kg b.w./d] [5)]	0.0430	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	25	See above
TER_{LT} [6)]	581	--

1 According to EFSA/2009/1438
2 Worst case PECsoil (max) value calculated for a twofold application of 150 g a.s./ha to cereals. For details see Sections B.8.2.1.1, Table 8.2.1.1.1.
3 Bioconcentration factor (BCF) = (0.84 + 0.012 x K_{OW}) / foc x Koc
4 PECworm = PECsoil x BCF
5 Daily dose = 1.28 x PECworm
6 TER_{LT} = NO(A)EL / Daily dose.
7 M-CA 2.7 lists logK_{OW} = 3.4. For K_{OW} value, see Section B.2.7/01, Wilbrand S. 2013c
8 Geometric mean K_{oc} of n=8 soils used

Food chain from fish to fish-eating mammals

The risk assessment for fish-eating mammals is based on the worst case PEC_{SW} (max. Step 1) derived from the environmental fate section (see section III CP B.8.2.5). The calculations and the resulting TER_{LT} values are presented in Table B.9.1.2.2-4.

Table B.9.1.2.2-4: Risk assessment for the active substance BAS 750 F concerning fish-eating mammals (tier 1) [1)]

Parameter	BAS 750 F	Reference
$PEC_{SW,}$ (max. step 1) [mg/L] [2)]	0.020592	III CP B.8.5, Tables 8.5-7 and 8.5-8
BCF fish (max. worst case)	385	III CA B.9.2.2/7, [redacted] 2015c
PEC_{fish} [mg/kg] [3)]	7.9279	--
Daily dose [mg/kg b.w./d] [4)]	1.1258	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	25	See above
TER_{LT} [5)]	22.21	--

1 According to EFSA/2009/1438
2 PEC_{SW} (max. Step 1) resulting from FOCUS Step 1 150g a.s./ha application on winter or spring cereals
3 $PEC_{fish} = PEC_{SW,}$ (max. step 1) x BCF
4 Daily dose = 0.142 x PEC_{fish}
5 TER_{LT} = NO(A)EL / Daily dose

In conclusion, according to the tier 1 risk assessment for earthworm- and fish-eating mammals, the TER values for BAS 750 F are above the trigger set by Commission regulation (EU) 546/2011, i.e. ≥ 5 for reproductive exposure, indicating a low risk for mammals from the use of BAS 750 01 F according to the proposed use pattern.

Risk for mammals through drinking water

Of the two drinking water risk assessment scenarios in EFSA/2009/1438, *i.e.* the leaf and the puddle scenario, the leaf scenario is not relevant for mammals. Consequently, the 'puddle scenario' will be considered for the application of BAS 750 01 F in cereals.

According to EFSA/2009/1438 no specific calculations of exposure and TER values 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 ($K_{oc} < 500$) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$). The ratio for the acute and reproductive endpoint for BAS 750 F (0.146 and 11.70, respectively) do not exceed the threshold value of 3000 as given by EFSA/2009/1438 for more sorptive substances ($K_{oc} \geq 500$), thus no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary (Table B.9.1.2.2-5).

Table B.9.1.2.2-5: Screening step for drinking water risk assessment in cereals-ratio of effective application rate to relevant endpoint for mammals

Parameter	BAS 750 F	Reference
K_{oc} (geometric mean)	3455.6 [6]	III CA B.8.5, Table 8.3.5
DT_{50} (soil) [d]	200	III CA B.8, Table 8.2
Number of applications	2	See above
Interval [d]	14	See above
MAF_m [1]	1.95	--
Max use rate [g/ha]	150	See above
AR_{eff} [g/ha] [2]	292.5	--
LD_{50} [mg/kg b.w.]	> 2000	See above
Ratio (acute) [3]	0.146	--
NO(A)EL [mg/kg b.w./d]	25	See above
Ratio (repro) [3]	11.70	--
Trigger [4]	3000	--
Drinking water assessment required [Yes/No] [5]	No	--

1 $MAF_m = (1-e^{-nki}) / (1-e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d].
2 AR_{eff} = Application rate (g/ha) x MAF_{mean}
3 Ratio of AR_{eff} and relevant toxicity endpoint
4 Trigger according to EFSA/2009/1438
5 Drinking water risk assessment is not necessary when trigger value is not exceeded.
6 Geometric mean K_{oc} of n=8 soils used

In conclusion, a quantitative drinking water risk assessment for the puddle scenario is not triggered for mammals, indicating a low risk for the intended use of BAS 750 01 F in cereals.

Metabolite assessment

The risk from metabolites of BAS 750 F is addressed in the bird chapter, M-B.9.1.1.2. Please refer to this part of the dossier for details.

Overall conclusion

In conclusion, all TER values for the dietary acute and long-term, and secondary poisoning risk assessments for BAS 750 01 F exceed the trigger value set by Commission regulation (EU) 546/2011 for acceptability, and the ratio of the effective application rate to the relevant toxicity endpoint indicates that a quantitative risk assessment for drinking water exposure is not required. This indicates a low risk for mammals from use of BAS 750 01 F according to the proposed use pattern.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

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

Report: B.9.3.1/1
[redacted] 2014a
BAS 750 01 F Rainbow trout, acute toxicity test
2014/1117112
Guidelines: OECD 203 (1992), EPA 850.1075
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F, batch no. FD-140113-0006; content of BAS 750 F: 98.9 g/L; density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*) aged approximately 3 months with average body length: 4.7 cm and average body weight: 1.32 g. Supplied by "Culture of Salmonidae fish", Zawoja, Poland.

Test design: Static system over 96 hours with 2 replicates per treatment. 10 fish per replicate (loading: 0.38 g fish/L), Mortality and symptoms of toxicity were assessed after 3, 6, 24, 48, 72 and 96 h after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, 0.09, 0.20, 0.43, 0.94, 2.06, 4.54 and 10 mg BAS 750 01 F/L (nominal). The contents of all aquaria were visually homogenous and transparent, except for 10 mg/l, which was homogenous but was slightly non-transparent. A reference test was performed with 3,5-dichloroaniline in a separate study performed in the laboratory in October 2014, and at 8 mg/L, 100% mortality was observed at 48h.

Preparation: Test solutions were prepared by appropriate amounts of the test substance in a glass crystaliser being mixed into 150mL of reconstituted water. The mixture was then introduced to aquaria with 35L reconstituted water for a concentration of 20 mg/L. All other test solutions were created by dilution of this stock solution.

Test conditions: Glass aquaria with lids and test volume 35L. The dilution water was reconstituted water (deionised water with stock solutions of reagent grade chemicals), which was continuously aerated prior to test initiation. No feeding of the test organisms took place over the test duration.

Temperature:	13.0-15.1°C;
pH:	6.64-7.68;
Oxygen concentration:	70%-99%;
Total hardness:	232-262 mg $CaCO_3$/L;
Conductivity:	609-707 µS/cm;
Photoperiod:	16 h light: 8 h dark.

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD-detection. The limit of quantification was 0.02 mg/L and the limit of detection was 0.01 mg/L.

Statistics: Probit analysis was used for calculation of LC_{50} and Fisher's Exact Binomial test with Bonferroni Correction for the determination of the NOEC ($\alpha = 0.05$). ToxRat professional 2.10 was used to conduct the statistical analysis.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 203 (1992)) were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen saturation (minimum 70%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning of the test, 48 h after start of exposure and at the end of the test. The measured concentrations of BAS 750 F ranged from 82.33% to 90.00%, 82.22% to 96.84% and from 82.47% to 94.79% of nominal at test initiation, 48 h after start of exposure and at the end of the test, respectively. As the measured concentrations confirmed correct application of the test substance and were maintained within ±20% of the nominal, the biological results are based on nominal concentrations.

After 96 hours of exposure no mortality was observed in the control and at the lowest test substance concentration of 0.09 mg BAS 750 01 F/L, whereas 35% and 80% mortality occurred at the test substance concentrations of 0.43 mg/L and 0.94 mg/L, respectively. At the test substance concentrations of 2.06 mg/L, 4.54 mg/L and 10 mg/L all fish were dead. After 96 hours of exposure, sublethal effects were observed at the test substance concentrations of 0.20 mg/L, 0.43 mg/L and 0.94 mg/L. The results are summarised in Table B.9.2.1/1-1.

Table B.9.2.1/1-1: Acute toxicity of BAS 750 01 F on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Control**	**0.09**	**0.20**	**0.43**	**0.94**	**2.06**	**4.54**	**10**
Mortality (96 h) [%]	0	0	10	35 *	80 *	100 *	100 *	100 *
Symptoms (96 h) #	none	none	U, R	U, R, P, E	U, R, P E	n.d.	n.d.	n.d.
Endpoints [mg BAS 750 01 F/L] (nominal)								
LC_{50} (96 h)	0.52 (95% confidence limits: 0.41-0.66)							
NOEC (96 h)	0.09							

* Statistically significantly different compared to the control

Symptoms after 96 h: U = unbalanced swimming behaviour, R = faulty respiratory function, P = non-typical pigmentation, E = loss of equilibrium

n.d. = not determined; all animals dead

III. CONCLUSION

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of BAS 750 01 F was 0.52 mg/L based on nominal concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an LC_{50} of 0.52 mg/L and a NOEC of 0.09 mg/L. The RMS notes the hardness of 262 mg $CaCO_3$/L exceeds the recommended levels of 250 mg $CaCO_3$/L and is not expected to have negatively affected the study. This is supported by all the validity criteria being met and no adverse effects observed in the controls. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint considered suitable for use in the risk assessment is:
LC_{50} of 0.52 mg formulation/L

Report: B.9.3.1/2
[redacted] 2015a
BAS 750 BS F (blank formulation of BAS 750 01 F) Rainbow trout, Acute toxicity test
2015/1001875
Guidelines: OECD 203 (1992), EPA 850.1075
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 BS F (blank formulation of BAS 750 01 F), batch no. FD-131216-0017 and contains no active substance.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), aged approximately 3.5 months with average body length 4.4 ± 0.3 cm and average body weight 1.07 ± 0.19 g. Supplied by "Culture of Salmonidae fish", Zawoja, Poland.

Test design: Static system of 96 hours with 2 replicates per treatment and 10 fish per replicate (loading: 0.31 g fish/L). Mortality and symptoms of toxicity were assessed after 3, 6, 24, 48, 72 and 96 h after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: 0 (control), 0.5, 1, 2, 4 and 8 mg BAS 750 BS F/L (nominal).

Preparation: The test substance was weighed separately for each test concentration into glass beakers to which 50 mL of deionised water was added, sonicated for 15 seconds and then added to the relevant aquarium with test medium. All aquaria were visually homogenous and transparent.

Test conditions: Glass aquaria with lids and a test volume of 35 L. The dilution water was reconstituted water (deionised water with stock solutions of reagent grade chemicals) which was aerated. No feeding of the test organisms took place over the test duration.

Temperature:	13.0-13.4°C;
pH:	7.09-7.61;
Oxygen concentration:	75-100% of air saturation value;
Total hardness:	235-250 mg $CaCo_3$/L;
Conductivity:	603-646 µS/cm;
Photoperiod:	16 h light: 8 h dark, with 30 min transition;

Analytics: No analytical verification of test substance concentrations.

Statistics: Probit analysis was used for calculation of LC_{50} and Fisher's Exact Binomial test with Bonferroni Correction for determination of the NOEC ($\alpha = 0.05$). ToxRat professional 2.10 was used to conduct statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 203 (1992)) were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen saturation (minimum 75%)

No analytical verification of test substance concentrations was conducted as the test substance was a blank formulation so no active substance was present. The biological results are based on nominal concentrations.

After 96 hours of exposure no mortality was observed in the control and at the lowest test substance concentration of 0.5 mg/L, whereas 5% mortality occurred at the test substance concentrations of 1 mg/L. At the three highest concentrations of 2, 4 and 8 mg/L all fish were dead. After 96 hours of exposure, sublethal effects were observed at the test substance concentration of 1 mg/L. The results are summarised in Table B.9.2.1/2-1.

Table B.9.2.1/2-1: Acute toxicity of BAS 750 BS F on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Control**	**0.5**	**1**	**2**	**4**	**8**
Mortality (96 h) [%]	0	0	5	100*	100*	100*
Symptoms (96 h) #	none	none	SP, FO	n.d.	n.d.	n.d.
Endpoints [mg BAS 750 BS F/L] (nominal)						
LC_{50} (96 h)	1.17					
NOEC (96 h)	0.5					

* Statistically significantly different compared to the control

\# Symptoms after 96 h: SP = unbalanced swimming behaviour, FO = faulty respiratory function

n.d. = not determined; all animals dead

III. CONCLUSION

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of BAS 750 BS F (blank formulation of BAS 750 01 F) was 1.17 mg/L based on nominal concentrations.

RMS Comment: This study is considered gratuitous and unnecessary vertebrate testing, and therefore an evaluation and conclusion on this study has not been completed. However this study has been considered further within the aquatic risk assessment.

Report: B.9.3.1/3
Turek T., 2015a
BAS 750 01 F *Daphnia magna*, acute immobilization test
2014/1117111

Guidelines: OECD 202 (2004), EPA 850.1010

GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F, batch no. FD-140113-0006; content of BAS 750 F 98.9 g/L; density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in-house culture, less than 24 hours old at test initiation and not first brood progeny.

Test design: Static system of 48 hours with 5 test concentrations plus a control, each with 4 replicates with 5 daphnids in each. Immobility was assessed after 24 and 48 hours.

Endpoints: NOEC, EC_{50} based on immobility of daphnids.

Test concentrations: Control, 0.63, 1.25, 2.5, 5.0, 10 mg BAS 750 01 F/L (nominal). The stock appeared visually homogenous and non-transparent. Potassium dichromate was used as a reference on a regular basis, last performed on December 2014 with a 48h LC_{50} of 0.51 mg/L.

Preparation: A stock solution of 10 mg/L was created by adding test medium to the 20.86 mg of the test substance to a volume of 2086mL. The stock solution and Additional test solutions were prepared by sequential dilution.

Test conditions: 150 mL glass beakers of test volume 100 mL with the dilution water "M7" (Elendt medium). Neither feeding nor aeration was performed over the course of the test.

pH	7.58-7.67;
Oxygen content:	95.0-99.3%;
Temperature:	19.5°C-20.6°C;
Photoperiod:	16 h light: 8 h dark

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD-detection. The limit of quantification was 0.02 mg/L and the limit of detection was 0.01 mg/L.

Statistics: Probit analysis was used for calculation of EC_{50} and Fisher's Exact Binomial test with Bonferroni Correction for determination of the NOEC ($\alpha = 0.05$). ToxRat professional 2.10 was the software used for statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- ≤10% control mortality (0%)
- ≥3 mg/L dissolved oxygen (minimum 95.0%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically detected concentrations for BAS 750 F ranged from 84.48% to 99.21% of the nominal concentration at test initiation and from 88.00% to 94.22% of nominal at test termination. As the measured concentrations confirmed correct application of the test

substance and were within ±20% of the nominal, the following biological results are based on nominal concentrations.

After 48 hours of exposure, 5%, 15% and 75% of the daphnids were immobile at the three lowest tested concentrations of 0.63 mg/L, 1.25 mg/L and 2.5 mg/L, respectively, whereas all daphnids were immobile at the two highest test substance concentrations of 5.0 mg/L and 10 mg/L. No immobility was observed in the control. For results see Table B.9.2.1/3-1.

Table B.9.2.1/3-1: Effect of BAS 750 01 F on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**0.63**	**1.25**	**2.5**	**5.0**	**10**
Immobility (24 h) [%] #	0	0	0	10	50	85
Immobility (48 h) [%]	0	5	15	75 *	100 *	100 *
Endpoints [mg BAS 750 01 F/L] (nominal)						
EC_{50} (48 h)	1.80 (95% confidence limits: 1.47-2.20)					
NOEC (48 h)	1.25					

* Statistically significantly different compared to the control
\# Statistically significant differences compared to the control were only determined for immobility data after 48 hours of exposure.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of BAS 750 01 F was 1.80 mg/L based on nominal concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an EC_{50} of 1.80 mg/L and a NOEC of 1.25 mg/L. The RMS notes that some water quality parameters such as hardness and conductivity were not reported, although as all the validity criteria were met and no adverse effects were observed in the controls, these omissions are not considered critical. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint considered suitable for use in the risk assessment is:
EC_{50} of 1.80 mg formulation/L

Report: B.9.3.1/4
Brzozowska-Wojoczek K., 2015 a
BAS 750 BS F (blank formulation of BAS 750 01 F) *Daphnia magna*, Acute immobilization test
2015/1177056
Guidelines: OECD 202 (2004)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 BS F (blank formulation of BAS 750 01 F), batch no. FD-131216-0017, and contains no active substance.

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates collected from in-house culture, less than 24 hours old at test initiation and not first brood progeny.
Test design:	Static system over 48 hours for 5 test concentrations plus control, each with 4 replicates with 5 daphnids in each. Immobility was assessed after 24 and 48 hours.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	0 (control), 2.0, 3.0, 4.5, 6.7, 10 mg BAS 750 BS F/L (nominal). Potassium dichromate was used as a reference on a regular basis, last performed on December 2014 with a 48h LC_{50} of 0.41 mg/L. The highest test concentration of 10 mg/L from which the other solutions were prepared was visually homogenous.
Preparation:	13.4 mg test substance was weighed into a glass crystaliser, and transferred by washing with Elendt M7 medium into a volumetric flask and filled to a volume of 1340mL to a concentration of 10 mg/L. All other test solutions were prepared by sequential dilution of the 10 mg/L solution.
Test conditions:	150 mL glass beakers with a 100mL test volume of dilution water "M7" (Elendt medium). Neither feeding nor aeration were performed over the duration of the test.
	Temperature: 21.0-21.7°C; pH: 7.17-7.46; Oxygen concentration: 8.5-9.1 mg/L; Photoperiod: 16 h light: 8 h dark;
Analytics:	No analytical verification of test substance concentrations.
Statistics:	Probit analysis was used for calculation of EC_{50} and Fisher's Exact Binomial test with Bonferroni Correction for determination of the NOEC ($\alpha = 0.05$). ToxRat professional 2.10 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- ≤10% control mortality (0%)
- ≥3 mg/L dissolved oxygen (minimum 8.5 mg/L)

No analytical verification of test substance concentrations was conducted as the test substance was a blank formulation so no active substance was present. The following biological results are based on nominal concentrations.

After 24 hours of exposure no immobility of daphnids was observed in the control and at test substance concentrations of up to and including 3.0 mg BAS 750 BS F/L. After 48 hours of exposure no immobility of daphnids was observed in the control. At the end of the test all daphnids were immobile at the highest test substance concentration of 10 mg/L. The results are summarised in Table B.9.2.1/4-1.

Table B.9.2.1/4-1: Effect of BAS 750 BS F on immobility of *Daphnia magna*

Concentration [mg/L] (nominal)	**Control**	**2.0**	**3.0**	**4.5**	**6.7**	**10**
Immobility (24 h) [%] [#]	0	0	0	35	40	45
Immobility (48 h) [%]	0	5	55*	85*	95*	100*
Endpoints [mg BAS 750 BS F/L] (nominal)						
EC_{50} (48 h)	3.14 (95% confidence limits: 2.72-3.59)					
NOEC (48 h)	2.0					

[#] Statistically significant differences compared to the control were only determined for immobility data after 48 hours of exposure.

* Statistically significantly different compared to the control

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of BAS 750 BS F (blank formulation of BAS 750 01 F) was 3.14 mg/L based on nominal concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an EC_{50} of 3.14 mg/L and a NOEC of 2.0 mg/L. This study has been considered further within the aquatic risk assessment. The RMS notes that some water quality parameters such as hardness and conductivity were not reported, although as all the validity criteria were met and no adverse effects were observed in the controls, these omissions are not considered critical. Additionally in the study report dissolved oxygen is incorrectly listed in percentage rather than in mg/L, as it is earlier in the report.

The agreed endpoint considered suitable for use in the risk assessment is:
EC_{50} of 3.14 mg blank formulation/L

Report: B.9.3.1/5
Turek T., 2015b
BAS 750 01 F *Pseudokirchneriella subcapitata SAG* 61.81 Growth inhibition test
2014/1117110
Guidelines: OECD 201 (2006), EPA 850.4500
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F, batch no. FD-140113-0006; content of BAS 750 F: 98.9 g/L; density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Unicellular green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; in-house culture; stock obtained from "Culture Collection of Algae“, Göttingen University, Germany.

Test design:	Static system over 96 hours with 6 test concentrations, each with 4 replicates per treatment plus a control with 8 replicates. Growth and cell morphology was assessed daily.
Endpoints:	NOEC, EC_{10} and EC_{50} with respect to growth rate and yield.
Test concentrations:	Control, 0.31, 1.0, 3.1, 10, 31, 100 mg/L (nominal). The stock solution was visually homogenous and non-transparent. A test with the reference substance 3,5-dichlorophenol was performed in May 2014 and had an E_rC_{50} of 2.56 after 72h.
Preparation:	151.60 mg of test substance was added to a flask and then AAP medium added to a volume of 1516mL. This stock solution was sequentially diluted with AAP medium to form the test solutions.
Test conditions:	250 mL Erlenmeyer glass flasks with 100mL test volume of test medium (AAP medium). Initial cell densities: 1×10^4 cells/mL; pH 7.13-7.22 (initiation) and 7.19-8.99 (termination); Temperature: 24.8-25.6 °C; Light intensity: 4243-4650 lux (continuous) Shaking: 90rpm constant mechanical shaking
Analytics:	Analytical verification of test substance concentrations was conducted using an HP-method with DAD-detection. The limit of quantification was 0.02 mg/L and the limit of detection was 0.01 mg/L
Statistics:	Probit analysis for determination of EC_x values for growth rate and yield. Statistically significant differences compared to the control ($\alpha = 0.05$) were determined using Williams Multiple Sequential t-test for yield data and Welch-t test with Bonferroni-Holm Adjustment for growth rate data. ToxRat professional 2.10 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 201 (2011)) were met:

- ≥16-fold control biomass increase over 72h (164-fold)
- ≤35% control section-by-section growth rate coefficient of variation (13.6%)
- ≤7% average specific growth rate coefficient of variation (1.8%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically detected concentrations of BAS 750 F ranged from 90.87% to 109.68% of the nominal concentration at test initiation and from 83.23% to 88.77% of nominal at test termination. As the measured concentrations confirmed correct application of the test substance and the concentrations were within ±20% of the nominal concentrations, the biological results are based on nominal concentrations.

No morphological effects on the algae were observed in the control and at test substance concentrations of up to and including the highest test substance concentration. Statistically significant differences for growth rate and yield compared to the control were observed at the five highest test substance concentrations after 72 h and 96 h of exposure. The effects on algal growth are summarised in Table B.9.2.1/5-1.

Table B.9.2.1/5-1: Effect of BAS 750 01 F on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**0.31**	**1.0**	**3.1**	**10**	**31**	**100**
Inhibition in 72 h (growth rate) [%] [#]	--	-2.9	6.6 *, a)	13.3 *, a)	59.2 *, a)	88.0 *, a)	91.3 *, a)
Inhibition in 72 h (yield) [%] [#]	--	-15.8	28.4 *, b)	48.7 *, b)	95.6 *, b)	99.4 *, b)	99.6 *, b)
Inhibition in 96 h (growth rate) [%]	--	0.2	4.8 *, a)	5.9 *, a)	43.0 *, a)	93.2 *, a)	94.3 *, a)
Inhibition in 96 h (yield) [%]	--	1.1	25.5 *, b)	30.0 *, b)	92.6 *, b)	99.9 *, b)	99.9 *, b)
Endpoints [mg BAS 750 01 F/L] (nominal)							
E_rC_{50} (72 h)	**8.45 (95% confidence limits: 7.39-9.67)**						
E_rC_{10} (72 h)	2.16 (95% confidence limits: 1.56-2.75)						
E_yC_{50} (72 h)	2.52 (95% confidence limits: 2.09-3.05)						
E_yC_{10} (72 h)	0.57 (95% confidence limits: 0.35-0.79)						
E_rC_{50} (96 h)	11.19 (95% confidence limits: 10.40-12.05)						
E_rC_{10} (96 h)	4.35 (95% confidence limits: 3.59-5.03)						
E_yC_{50} (96 h)	3.65 (95% confidence limits: 2.93-4.57)						
E_yC_{10} (96 h)	0.92 (95% confidence limits: 0.51-1.31)						
NOE_rC (72h)	0.31 mg/L						
NOE_rC (96h)	0.31 mg/L						
NOE_yC (72h)	0.31 mg/L						
NOE_yC (96h)	0.31 mg/L						

[#] Negative values indicate stimulated growth compared to the control.
* Statistically significantly different compared to the control.
a) Welch-t test with Bonferroni-Holm Adjustment, $\alpha = 0.05$.
b) Williams Multiple Sequential t-test, $\alpha = 0.05$.

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} (96 h) of BAS 750 01 F was determined to be 11.19 mg/L based on nominal concentrations. After 72 hours of exposure, the E_rC_{50} (72 h) was determined to be 8.45 mg/L (nominal).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an E_rC_{50} (72 h) of 8.45 mg/L and an E_rC_{10} (72 h) of 2.16 mg/L. The RMS notes that the temperature exceeded the recommended (OECD 201 (2011)) upper limit of 24°C at all points during the study although at most by 1.6°C, that light intensity dipped marginally below the recommended minimum of 4440lux (minimum 4243lux) and that pH increased by more than 1.5 (7.22-8.99 in the control) over the course of the study. Given that all the validity criteria have been met and no negative effects have been observed in the controls, these deviations are not expected to have adversely affected the study. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint considered suitable for use in the risk assessment is:
E_rC_{50} (72 h) of 8.45 mg formulation/L

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

Based on the data obtained with the blank formulation it can be demonstrated that the increased acute toxicity of the formulation BAS 750 01 F is a result of co-formulants. Concurrent exposure to active substance and co-formulants is expected to be short lived due to the fast degradation of most of the formulation constituents (most of the co-formulants are readily biodegradable according to OECD criteria, see Doc J) and due to physical separation from the active substances in soil and in natural water. Further consideration of the co-formulants and the blank formulation studies is presented in the aquatic risk assessments below.

Chronic formulation testing and/or other consideration for the chronic risk assessment is therefore not necessary.

B.9.3.3. Further testing on aquatic organisms

No additional testing on aquatic organisms was conduction and no further data is required.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

B.9.4.1 Active substance

The endpoints from studies with the active substance are presented in Table B.9.4.1-1 below have been evaluated, assessed and considered suitable for use within risk assessments. The study summaries and evaluation of these studies are presented in III CA B.9.4.

Commission Regulation (EU) 283/2013 and 284/2013 require estimates of EC_x (e.g. EC_{10}, EC_{20}) together with the NOEC for chronic studies. If appropriate, an EC_x is given in the study summaries (chapter III CA B.9.4) or a respective justification. Where it is possible for both the NOEC and EC_{10} to be calculated for chronic studies, the EC_{10} value has been used in preference as per the latest aquatic guidance document (EFSA Journal 2013;11(7):3290).

Table B.9.4.1-1: List of studies and endpoints for aquatic organisms exposed to the active substance (BAS 750 F)

Organism	Substance	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked sediment study)	Reference
Acute toxicity to fish				
Oncorhynchus mykiss	BAS 750 F	96 h LC_{50}	**0.532**	III CA B.9.4.1.1/1, [redacted] 2014a
Cyprinus carpio		96 h LC_{50}	1.126	III CA B.9.4.1.1/2, [redacted] 2015c
Danio rerio, (Syn. *Brachydanio rerio*)		96 h LC_{50}	0.906	III CA B.9.4.1.1/3, [redacted] 2015a
Cyprinodon variegatus		96 h LC_{50}	0.761	III CA B.9.2.1.1/4, [redacted] 2014a

Organism	Substance	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked sediment study)	Reference
Chronic toxicity to fish				
Cyprinodon variegatus (ELS)	BAS 750 F	35 d NOEC	0.147	III CA B.9.2.2/1, [redacted], 2015a
Danio rerio (ELS)		36 d NOEC	**0.027**	III CA B.9.2.2/2, [redacted] *et al.*, 2015b
Endocrine disruption to fish				
Danio rerio (FSDT)	BAS 750 F	69 d NOEC	≥ 0.045	III CA B.9.2.3/1, [redacted] 2015a
Acute toxicity to aquatic invertebrates				
Daphnia magna	BAS 750 F	48 h EC_{50}	**0.944**	III CA B.9.2.4.1/1, Brzozowska., 2014a
Americamysis bahia #		48 h LC_{50} [2] 96 h LC_{50}	1.53 1.30	III CA B.9.2.4.1/2, VanHooser, 2014a
Crassostrea virginica		96 h EC_{50}	0.947	III CA B.9.2.4.1/3, VanHooser, 2014b
Chronic toxicity to aquatic invertebrates				
Daphnia magna	BAS 750 F	21d NOEC 21 d EC_{10}	0.0091 **0.0161**	III CA B.9.2.5/1, Janson, 2014a
Daphnia longispina		21d NOEC 21 d EC_{10}	0.0342 0.0564	III CA B.9.2.5/2, Janson, 2014b
Daphnia pulex		21d NOEC 21 d EC_{10}	0.0276 0.0567	III CA B.9.2.5/3, Janson, 2015a
Americamysis bahia #		28d NOEC	≥ 0.0132	III CA B.9.2.5/4, Dinehart, 2016a
Acute/ sub-chronic toxicity to sediment dwelling aquatic invertebrates				
Chironomus dilutes (spiked sediment)	BAS 750 F	10d NOEC	7.08 mg /kg dry sediment	III CA B.9.2.6/1, Clark, 2015a
		10d EC_{50}	> 96 mg /kg dry sediment	
Hyalella azteca (spiked sediment)		10d NOEC	≥ 100 mg /kg dry sediment	III CA B.9.2.6/4, Clark, 2015b
		10d EC_{50}	> 100 mg /kg dry	

Organism	Substance	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked sediment study)	Reference
			sediment	
Leptocheirus plumulosus (spiked sediment)		10d NOEC	≥ 95 mg /kg dry sediment	III CA B.9.2.6/5, Clark, 2015c
		10d LC_{50}	> 95 mg /kg dry sediment	
Chronic toxicity to sediment dwelling aquatic invertebrates				
Chironomus riparius (spiked sediment)	BAS 750 F	28 d NOEC	≥ 1.158 mg/kg dry sediment	III CA B.9.2.6/3, Backfisch &Weltje, 2015b
Algae [1)]				
Pseudokirchneriella subcapitata	BAS 750 F	72 h E_rC_{50}	1.352	III CA B.9.2.7.1/1, Brzozowska, 2014b
		72 h NOE_rC 72 h E_rC_{10}	0.103 0.904	
		72 h E_yC_{50}	0.777	
		72 h NOE_yC 72 h E_yC_{10}	<0.103 0.215	
Skeletonema costatum [#]		72 h E_rC_{50}	**0.679**	III CA B.9.2.7.1/2, Bergfield, 2015a
		72 h NOE_rC 72 h E_rC_{10}	0.0985 0.373	
		72 h E_yC_{50}	0.479	
		72 h NOE_yC 72 h E_yC_{10}	0.0985 0.257	
Navicula pelliculosa		72 h E_rC_{50}	1.347	III CA B.9.2.7.1/3, Bergfield, 2015b
		72 h NOE_rC 72 h E_rC_{10}	0.303 0.478	
		72 h E_yC_{50}	0.671	
		72 h NOE_yC 72 h E_yC_{10}	0.303 0.351	
Anabaena flos-aquae		72 h E_rC_{50}	> 3.08	III CA B.9.2.7.1/4, Bergfield, 2015c
		72 h NOE_rC 72 h E_rC_{10}	≥ 3.08 >3.08	
		72 h E_yC_{50}	> 3.08	
		72 h NOE_yC 72 h E_yC_{10}	≥ 3.08 >3.08	

Organism	Substance	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked sediment study)	Reference
Macrophytes [1)]				
Lemna gibba	BAS 750 F	7 d E_rC_{50}	**> 2.017**	III CA B.9.2.8/1, Swierkot, 2014a
		7 d NOE_rC	≥ 2.017	
		7d E_rC_{10}	> 2.017	
		7 d E_yC_{50}	> 2.017	
		7d NOE_yC	≥ 2.017	
		7d E_rC_{10}	> 2.017	
Bioconcentration				
Oncorhynchus mykiss (BCF; 14d uptake, 7 d depuration)	BAS 750 F	BCF_{KLg} (whole fish)	385	III CA B.9.2.2/3, [redacted] 2015c

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and macrophytes), only the relevant endpoint(s) is used in the (tier 1) risk assessment.

Abbreviations: ELS = early life stage; FSDT = fish sexual development test; BCF_{KLg} = growth corrected kinetic bioconcentration factor normalized to 5% lipid content;

[#] Estuarine/Marine species.

[1)] In accordance to the EFSA Aquatic Guidance Document (EFSA 2013), only the EC_{50} values determined for the more relevant endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers if both "growth rate" and "yield / biomass" endpoints are available.

[2] The applicant has argued for a 48h endpoint to be comparable to that of the 48h *Daphnia* study. In the absence of clear guidance, both 96h and 48h endpoints have been presented

B.9.4.2 Metabolites of BAS 750 F

According to Section III CA B.8.2.1 several major metabolites have been identified as occurring in the water phase. These metabolites are 1,2,4-triazole (=M750F001), M750F003, M750F005, M750F006, M750F007 and M750F008 and the endpoints are summarised in Table B.9.4.2-1.

It was determined that the metabolites 1,2,4-triazole (=M750F001), M750F003 M750F005, M750F006, M750F007 and M750F008 were also located in the sediment (III CP B.8.4), and therefore toxicity studies have been carried out on these metabolites. Toxicity studies were submitted for some of the following endpoints, whilst QSARs were used for some fish endpoints in order to minimise the amount of vertebrate testing.

Table B.9.4.2-1: List of studies and endpoints for aquatic organisms exposed to the metabolites of BAS 750 F

Organism	Endpoint	Value [mg/L] (except sediment endpoint of spiked sediment study)	Reference
1,2,4-triazole (Reg. No. 87084; M750F001)			
Fish			

Organism	Endpoint	Value [mg/L] (except sediment endpoint of spiked sediment study)	Reference
Oncorhynchus mykiss	96 h LC_{50}	498	EFSA Scientific Report (2008) 138, 1-80
	28 d NOEC	3.2	EFSA Scientific Report (2008) 138, 1-80
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	> 100	EFSA Scientific Report (2008) 138, 1-80
Algae			
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50}	22.5	EFSA Scientific Report (2008) 138, 1-80
M750F003 (Reg. No. 5924326)			
Fish			
QSAR Data	96 h LC_{50}	> 100	III CA B.9.12
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	> 100	III CA B.9.4.4.2/5 Haerthe N., 2016,
Algae			
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50}	> 100	III CA B.9.4.7.2/5 Backfisch K., 2016
Sediment-dwelling aquatic organisms			
Chironomus riparius (spiked sediment study)	28 d NOEC	≥ 1.944 mg/kg dry sediment	III CA B.9.4.6/2, Backfisch & Weltje, 2015a
M750F005 (Reg. No. 6003433)			
Fish			
QSAR Data	96 h LC_{50}	11.3	III CA B.9.12
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	> 8.58	III CA B.9.4.4.2/3, Rzodeczko, 2015d
Algae			
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50}	> 8.57	III CA B.9.4.7.2/4, Rzodeczko, 2016b
M750F006 (Reg. No. 5863469)			
Fish			
Oncorhynchus mykiss	96 h LC_{50}	6.2	III CA B.9.4.1.2/2, [redacted] 2016

Organism	Endpoint	Value [mg/L] (except sediment endpoint of spiked sediment study)	Reference
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	4.42	III CA B.9.4.4.2/2, Rzodeczko, 2015c
Algae			
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50}	1.42	III CA B.9.4.7.2/3, Rzodeczko, 2016a
M750F007 (Reg. No. 6003432)			
Fish			
Oncorhynchus mykiss	96 h LC_{50}	> 7.20	III CA B.9.4.1.2/1, [redacted] 2015b
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	> 10	III CA B.9.4.4.2/1, Backfisch & Härthe, 2015a
Algae			
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50}	> 10	III CA B.9.4.7.2/1, Backfisch, 2015a
M750F008 (Reg. No. 6010286)			
Fish			
QSAR Data	96 h LC_{50}	> 1.96	III CA B.9.12
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	> 8.07	III CA B.9.4.4.2/4, Rzodeczko, 2015e
Algae			
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50}	4.08	III CA B.9.4.7.2/2, Brzozowska-Wojoczek, 2015a

[1)] In accordance to the EFSA Aquatic Guidance Document (EFSA 2013), only the EC_{50} values determined for the more relevant endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers if both "growth rate" and "yield / biomass" endpoints are available.

B.9.4.3 BAS 750 01 F: The formulated product of BAS 750 F

As the intended use of this product could result in direct exposure of water via spray drift and the toxicity cannot be predicted for the active substance, the risk posed by the formulated product must be considered. Testing on fish, *Daphnia* and algae with the formulated product has been undertaken. Presented in Table B.9.4.3-1 is a comparison of the active substance and formulated product endpoints.

Table B.9.4.3-1: Comparison of toxicity of BAS 750 F (a.s.) and BAS 750 01 F (f.p.)

Organism	Substance	Endpoint	Value [mg/L]	Reference
Acute toxicity to fish				

Organism	Substance	Endpoint	Value [mg/L]	Reference
Oncorhynchus mykiss	BAS 750 F	96 h LC_{50}	0.532	III CA B.9.4.1/1, [redacted], 2014a
	BAS 750 01 F (f.p.)	96 h LC_{50}	0.52	III CP B.9.3.1/1 [redacted] 2014a
	BAS 750 01 F (expressed as a.s. in f.p.)[1]	96 h LC_{50}	0.0524	
	BAS 750 BS F[2]	96 h LC_{50}	1.17	III CP B.9.3.1/2 [redacted] A., 2015a
Acute toxicity to aquatic invertebrates				
Daphnia magna	BAS 750 F	48 h EC_{50}	0.944	III CA B.9.4.4/1, Brzozowska., 2014a
	BAS 750 01 F (f.p.)	48 h EC_{50}	1.80	III CP B.9.3.1/3 Turek T., 2015a
	BAS 750 01 F (expressed as a.s. in f.p.)[1]	48 h EC_{50}	0.1813	
	BAS 750 BS F[2]	48 h EC_{50}	3.14	III CP B.9.3.1/4 Brzozowska-Wojoczek K., 2015 a
Toxicity to algae				
Skeletonema costatum	BAS 750 F	72 h E_rC_{50}	0.679	III CA B.9.4.6/7, Bergfield, 2015a
Pseudokirchneriella subcapitata		72 h E_rC_{50}	1.352	III CA B.9.4.7.1/1, Brzozowska, 2014b
	BAS 750 01 F (f.p.)	72 h E_rC_{50}	8.45	III CP B.9.3.1/5 Turek T., 2015b
	BAS 750 01 F (expressed as a.s. in f.p.)[1]	72 h E_rC_{50}	0.8510	

[1]Calculated based upon a density of 0.993g/cm[3]

[2] This study was carried out with the blank formulation, i.e. the formulation without the active substance.

The above acute data indicate that the toxicity of the formulated product is greater than that of the active substance for both fish and aquatic invertebrates, i.e. the LC_{50} or EC_{50} for the formulated product when expressed as active substance is lower than the corresponding LC_{50} or EC_{50} for the active substance. Based upon the active substance content of the formulated product, the formulated product is 10.15 times more toxic than BAS 750 F for fish, 5.2 times more toxic for aquatic invertebrates and of comparable toxicity for algae (1.25 times less toxic, although it is noted a different, less sensitive species was tested)[4].

[4] In order to determine if the formulation increases toxicity a factor of 3 has been used, i.e. if the difference between the formulation and a.s. endpoints when both expressed as a.s. are less than or equal to 2, then it is concluded that the formulation doesn't enhance the toxicity. The factor of 3 has been taken from SANCO/10597/2003 –rev. 10.1 13 July 2012 GUIDANCE DOCUMENT ON THE ASSESSMENT OF THE EQUIVALENCE OF TECHNICAL MATERIALS OF SUBSTANCES REGULATED UNDER Regulation (EC) No 1107/2009

Data have been submitted on the toxicity of the blank formulated product that indicates that the co-formulants exhibit some toxicity to both aquatic life; the blank formulation has an acute toxicity to fish of a 96 h LC_{50} of 1.1 mg/L (B.9.3.1/2), and an acute toxicity to *Daphnia* of a 48 h EC_{50} of 3.14 mg/L (B.9.3.1/4).

As the endpoint from the formulated product based on the active substance content is more toxic than the active substance alone for fish and *Daphnia magna*, the risk assessments have been conducted with both a.s. and formulated product endpoints.

For STEPs 1 and 2, all RACs have been presented and hence the risk assessed; whilst at STEP 3, only those that had failures at STEP 1 and 2 are considered. For STEPs 1 and 2 data on the toxicity of the active substance and formulation have been used, whereas in considering the risk at STEP 3, it has been assumed that if the exposure will be via spray drift then the risk will be based on the endpoint from the formulated product. However, if the exposure is either drainflow or runoff driven, then the data from the a.s. will be used. The rationale for this is that co-formulants, which are of known toxicity (see blank formulated product study endpoints), are not expected to remain with the active substance in runoff or drainflow dominated scenarios. It should however be noted that a spray drift assessment has also been carried out for those scenarios where the risk is drainflow or runoff driven.

According to the data requirements (Commission Regulation (EU) No 284/2013), chronic studies on fish and invertebrates for the formulated product should only be conducted where it is not possible to extrapolate from data obtained in the corresponding studies on the a.s. (i.e. the PPP is more acutely toxic than the a.s. by a factor of 10), unless it is demonstrated that exposure will not occur. However, if the applicant demonstrates that the increased acute toxicity of the preparation is a result of co-formulants that will rapidly disappear and latency of effects is not to be expected, the RA can be based on the data for the a.s. and a chronic study with the PPP is deemed not necessary (EFSA Journal 2013;11(7):3290).

The acute toxicity of the active substance to fish when tested in the formulated product is 10.15 times greater than the active substance when it is tested on its own, i.e. marginally above the recommended factor of 10, hence further consideration is required.

For fish the LC_{50} is 1.17 mg/L for the blank formulation compared to an LC_{50} of 0.52 mg/L for the formulated product and an LC_{50} of 0.532 mg a.s./L for the a.s. It is, therefore, the view of the RMS that the blank formulated product is potentially contributing to the increased toxicity; it is also plausible that there is some additive or synergistic effects occurring between the active substance and the co-formulants. .

In addition to the data on the toxicity of the blank formulated product, it is noted that all the co-formulants bar one are rapidly degradable and therefore not relevant on the chronic scale. Further details on the formulated product are presented in the confidential section (Volume 4). However there is no environmental toxicity data presented for the non-rapidly degradable co-formulant. Therefore further consideration of the toxicity of the co-formulants is required.

Rapidly degradable co-formulants with toxicity ranging between 1-10 mg/L compose 31.22%w/w of the formulated product, whilst the less toxic co-formulants (10-100 mg/L) compose 38.20% w/w with the active substance making up 10.44% w/w. The non-rapidly degradable co-formulant represents the remaining 20.14% w/w, but is itself a mixture of a polymer of unknown toxicity and a rapidly degradable solvent. The rapidly degradable solvent has a LC_{50} of 14.8 mg/L for fish.

The toxicity of the active substance when tested in the formulation is less than 10 for *Daphnia magna* and hence there is no requirement to consider if chronic toxicity of the formulation are required.

Based upon the above data, it is considered that the co-formulants exert some toxicity and it is plausible that this is contributing to the higher toxicity of the formulation compared to the a.s. In addition there is evidence the majority of the co-formulants in terms of percent as weight for weight

are rapidly biodegradable, therefore it is the view of the RMS that chronic fish toxicity data with the formulation is not required.

B.9.4.4 Exposure to the active substance

The critical use pattern considered in the risk assessment below is presented in Table B.9.4.4-1.

Table B.9.4.4-1: Proposed use pattern considered for the risk assessment

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				BAS 750 F [kg a.s./ha]	BAS 750 01 F [L product/ha]
Cereals (winter/spring)	30-69	1-2	21	0.150	1.50

A stepwise approach has been followed starting with simple worst-case assumptions in the first two STEPs and proceeding to more realistic worst-case conditions in the third STEP and adding spray drift mitigation (*i.e.* no-spray buffer zones) and runoff mitigation (*i.e.* non-sprayed vegetated filter strips) in the fourth STEP of the exposure assessment.

Regarding FOCUS STEP 1 and 2, the calculated PEC values for application in winter cereals also cover application in spring cereals III CP B.8.5. Overall, only worst-case PECs either resulting from calculations for single or multiple applications at the proposed use rates of 100 g a.s./ha or 150 g a.s./ha are presented and used for risk calculations. For full details of the assumptions used in the exposure calculations, please see III CP B.8.2.5.

In addition to the calculations for the active substance, PEC_{SW} calculations for the formulated product BAS 750 01 F have been determined. These PEC values are only relevant for assessing the risk immediately after the entry of the formulated product into the water body (PEC_{ini}), and will be used with the toxicity endpoints derived from studies with the formulated product (i.e. those presented in Table B.9.4.3-1.

Risk to aquatic organisms from the active substance

As per EFSA Aquatic Guidance Document (EFSA Journal 2013;11(7):3290), assessment of the risk of the active substance will be undertaken using the appropriate PEC value for the proposed use of BAS 750 01 F. RAC values will be compared to the relevant PEC in a tiered process for both acute and long-term risk to aquatic organisms. The RACs to be used in the risk assessments are presented in Table B.9.4.4-2 below.

The most sensitive endpoint for each species has been used in calculating the RAC, with the exception of the chronic endpoint for the aquatic invertebrates. In this case, the most sensitive endpoint is an unbounded NOEC for *Americanamysis bahia*, although an EC_{10} for *Daphnia magna* that is not unbounded is available as the next most sensitive endpoint. The acute data indicates that *Daphnia magna* are the more sensitive species, so there is no reason to expect that the chronic *Americanamysis bahia* endpoint will be more sensitive than the *Daphnia magna* chronic endpoint, and there is no indication in the initiation of any effects at the top dose of the *Americanamysis bahia* study, so the endpoint for *Daphnia magna* has been used in the risk assessments.

Table B.9.4.4-2: Regulatory acceptable concentrations (RAC) for BAS 750 F for each organism group for the use of BAS 750 01 F in spring and winter cereals

Test species	***O. mykiss***	***D. rerio***	***D. magna***	***D. magna***	***C. riparius***	***S. costatum***	***L. gibba***
Endpoint [µg/L or µg/kg sediment]	LC_{50}	NOEC	EC_{50}	EC_{10}	NOEC	E_rC_{50}	E_rC_{50}
	532	27	944	16.1	≥ 1158	679	> 2017
AF	100	10	100	10	10	10	10
RAC (µg/L)	**5.32**	**2.7**	**9.44**	**1.61**	**≥ 115.8**	**67.9**	**> 201.7**

AF: Assessment factor

In addition and as discussed above, the endpoints for the formulated product (when expressed as active substance) are more toxic than the equivalent active substance endpoints and hence these will be used as outlined above. Presented in Table B.9.4.4-2 are the RACs based on the formulation data.

Table B.9.4.4-2: Regulatory acceptable concentrations (RAC) for BAS 750 F for each organism group for the use of BAS 750 01 F in spring and winter cereals

Test species	***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint [µg/L]	LC_{50}	EC_{50}	E_rC_{50}
	52.4	181.3	851
AF	100	100	10
RAC (µg/L)	**0.524**	**1.813**	**85.1**

AF: Assessment factor

Risk to aquatic organisms

The risk to aquatic organisms aquatic is presented below. The critical RAC for the active substance, BAS 750 F is the chronic RAC of 1.0 µg/L for aquatic invertebrates. Due to the issues highlighted above regarding the toxicity of the formulated product compared to the a.s., the endpoints from the studies with the formulated product have also been presented. The critical formulation RAC is 0.524 µg/L, which is for the acute toxicity to fish. If the risk is found to be high, i.e. the PEC_{SW} greater than the RAC, the risk to the next lowest RAC will be assessed and so on until there is a low risk presented at STEP 1.

STEP 1

Table B.9.4.4-3: Risk for BAS 750 F using the worst-case FOCUS STEP 1 $PEC_{SW, max}$ values

Test substance	**BAS 750 F**						**BAS 750 01 F (expressed as a.s. in f.p.)**		
Test species	***O. mykiss***	***D. rerio***	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint [µg/L]	LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	LC_{50}	EC_{50}	E_rC_{50}
	532	27	944	16.1	679	>2017	52.4	181.3	851
AF	100	10	100	10	10	10	100	100	10
RAC (µg/L)	5.32	2.7	9.44	1.61	67.9	>201.7	0.524	1.813	85.1
$PEC_{SW, max}$ [µg/L]	**20.592**	**20.592**	**20.592**	**20.592**	20.592	20.592	**20.592**	**20.592**	**20.592**

PECs shown in **bold** are greater than the RAC

* PEC values have been calculated for application in winter cereals, also covering application in spring cereals.

Using worst-case STEP 1 PEC values, there is a potential acute and chronic risk to fish and aquatic invertebrates. As regards to the formulated product data, these indicate a potential risk to both fish and aquatic invertebrates as well as a risk to algae. As the RAC for *S. costatum* was greater than the PEC_{SW}, the risk to algae is low. Additionally, as the RAC for *L. gibba* is greater than that for algae, the risk to aquatic plants can also be concluded as low.

STEP 2

Table B.9.4.4-4: Risk for BAS 750 F using the worst-case FOCUS STEP 2 $PEC_{SW, max}$ values for both North and South Europe

Test substance			**BAS 750 F**				**BAS 750 01 F (expressed as a.s. in f.p.)**	
Test species			***O. mykiss***	***D. rerio***	***D. magna***	***D. magna***	***O. mykiss***	***D. magna***
Endpoint [µg/L]			LC_{50}	NOEC	EC_{50}	EC_{10}	LC_{50}	EC_{50}
			532	27	944	16.1	52.4	181.3
AF			100	10	100	10	100	100
RAC (µg/L)			5.32	2.7	9.44	1.61	0.524	1.813
$PEC_{SW, max}$ [µg/L]*	South Europe	Single application	3.156	**3.156**	3.156	**3.156**	**3.156**	**3.156**
		Multiple application	**6.080**	**6.080**	6.080	**6.080**	**6.080**	**6.080**
	North Europe	Single application	1.749	1.749	1.749	**1.749**	**1.749**	1.749
		Multiple application	3.332	**3.332**	3.332	**3.332**	**3.332**	**3.332**

PECs shown in **bold** are greater than the RAC
* PEC values have been calculated for application in winter cereals, also covering application in spring cereals.

Using worst-case STEP 2 PEC values, on the basis of the a.s. derived data, there is a possible chronic risk posed to fish and aquatic invertebrates and a possible acute risk posed to fish. Using the data derived using the formulated product, there is a potential acute risk to fish and aquatic invertebrates. As the formulated product RAC for *P. subcapitata* was greater than the PEC_{SW}, the risk to algae from the formulation is low.

STEP 3

For STEP 3, all RACs that failed at STEP 2 have been considered in order of the most sensitive RAC first. If there are any failing scenarios, the next most sensitive RAC must also be considered at STEP 3. For the RAC where all the scenarios pass at STEP 3, all less sensitive RACs are not required to be considered further as they will be covered by the RAC that passes all scenarios.

Due to the difference in toxicity between the formulated product and the a.s. (see B.9.4.3), it is necessary to determine which is the appropriate endpoint to use in the risk assessment. The endpoints from the studies conducted with the formulated product are considered to be relevant where spray drift is the major route of exposure. As the endpoint from the studies conducted with the formulated product are lower they will also cover the risk from any subsequent drainflow or runoff However, when the exposure is due to either drainflow, runoff or accumulation, it is considered more relevant to use the a.s. data. In light of this, the following is proposed:

- Spray drift values following application of the formulated product have been calculated based on the maximum initial exposure due to spray drift (III CP B.8.5). The calculation of PEC_{SW} values by spray drift is equivalent for all scenarios that share the same body of water. Therefore only three PEC_{SW} values have been determined. These PEC values cover the ditch, pond and stream scenarios and are appropriate for comparison against the RAC for the formulated product
- Active substance RACs that failed STEP 2 have been considered with the PEC_{SW} values for all STEP 3 scenarios relevant for Winter and Spring cereals

The above issue is only relevant for the acute risk to fish and *Daphnia magna* and is not relevant to the long-term risk to fish or *Daphnia magna*.

Presented in Table B.9.4.4-5 is the risk assessment for the acute risk to fish from the formulated product.

Table B.9.4.4-5: Acute risk assessment for *O. mykiss* exposed to BAS 750 01 F expressed as BAS 750 F in f.p. using the spray drift only values following application in spring and winter cereals

Test organism	Application rate BAS 750 f (g/ha)	RAC [µg/L]	Scenario	$PEC_{SW, ini}$ [µg/L]
O. mykiss	150	0.524	Ditch	**0.9637**
			Pond	0.0329
			Stream	**0.7152**

PECs shown in **bold** are greater than the RAC

As there were failures for the acute risk to fish based upon BAS 750 01 F expressed as the concentration of a.s. in the formulated product consideration of the second most sensitive RAC at STEP 3 (chronic risk to aquatic invertebrates) is required and has been considered in Table B.9.4.4-6 below. The unresolved acute risk to fish from the formulated product has been considered under the STEP 4 section.

Table B.9.4.4-6: Long-term risk assessment for *D. magna* exposed to BAS 750 F using the worst-case (tier 1) FOCUS STEP 3 PEC_{SW} values following application in spring and winter cereals

Test organism	RAC [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L] *
Spring cereals					
D. magna	1.61	D1	ditch	Multiple	**1.703**
			stream	Single	0.846
		D3	ditch	Single	0.948
		D4	pond	Multiple	0.089
			stream	Single	0.775
		D5	pond	Multiple	0.048
			stream	Single	0.796
		R4	stream	Single	0.627
Winter Cereals					
D. magna	1.61	D1	ditch	Multiple	1.558
			stream	Single	0.841
		D2	ditch	Multiple	**2.456**
			stream	Multiple	1.533
		D3	ditch	Single	0.948
		D4	pond	Multiple	0.075
			stream	Single	0.791
		D5	pond	Multiple	0.052
			stream	Single	0.756
		D6	ditch	Single	0.952
		R1	pond	Multiple	0.153
			stream	Multiple	0.684

Test organism	RAC [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L] *
		R3	stream	Single	0.877
		R4	stream	Single	0.627

PECs shown in **bold** are greater than the RAC
* Tier 1 (standard parametrization)

As STEP 3 values still indicate an unacceptable risk in the D1 multiple application scenario for Spring cereals, and D2 ditch multiple applications for Winter cereals, further consideration at STEP 4 is required and has been considered in the STEP 4 section. In addition, the risk posed to other groups that failed the STEP 2 risk assessment must also be considered here in the STEP 3 section.

Presented in Table B.9.4.4-7 is the risk assessment for the acute risk to *Daphnia magna* from the formulated product.

Table B.9.4.4-7: Acute risk assessment for *D. magna* exposed to BAS 750 01 F expressed as BAS 750 F in f.p. using the spray drift only values following application in spring and winter cereals

Test organism	Application rate BAS 750 f (g/ha)	RAC [µg/L]	Scenario	$PEC_{SW, ini}$ [µg/L]
D. magna	150	1.813	Ditch	0.9637
			Pond	0.0329
			Stream	0.7152

PECs shown in **bold** are greater than the RAC

The risk assessment results an acceptable risk from the formulated product to aquatic invertebrates at STEP 3. As the RAC for the active substance passed at STEP 2 no further consideration of the risk to acute aquatic invertebrates is required. However the chronic risk to fish was not resolved at STEP 2 and therefore requires consideration at STEP 3.

Presented in Table B.9.4.4-8 is the risk assessment for the long-term/chronic risk to fish from the active substance.

Table B.9.4.4-8: Fish long-term risk for BAS 750 F using the FOCUS STEP 3 PEC_{SW} values following application in spring and winter cereals

Test organism	RAC [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L] *
Spring cereals					
D. rerio	2.7	D1	ditch	Multiple	1.703
			stream	Single	0.846
		D3	ditch	Single	0.948
		D4	pond	Multiple	0.089
			stream	Single	0.775
		D5	pond	Multiple	0.048
			stream	Single	0.796
		R4	stream	Single	0.627

Test organism	RAC [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L] *
Winter cereals					
D. rerio	2.7	D1	ditch	Multiple	1.558
			stream	Single	0.841
		D2	ditch	Multiple	2.456
			stream	Multiple	1.533
		D3	ditch	Single	0.948
		D4	pond	Multiple	0.075
			stream	Single	0.791
		D5	Pond	Multiple	0.052
			stream	Single	0.756
		D6	ditch	Single	0.952
		R1	pond	Multiple	0.153
			stream	Multiple	0.684
		R3	stream	Single	0.877
		R4	stream	Single	0.627

* Tier 1 (standard parametrization)

The RAC value for BAS 750 F exceeds the $PEC_{SW, max}$ based on FOCUS STEP 3 calculations, indicating low chronic risk to fish following the proposed uses of BAS 750 01 F. Additionally as the *O. mykiss* RAC for the acute risk to fish is greater than the *D. rerio* RAC for the chronic risk to fish, the risk to acute fish can be concluded as being low. However, for completeness, the STEP 3 risk assessment for the acute risk to fish from the active substance is presented below.

Presented in Table B.9.4.4-9 is the risk assessment for the acute risk to fish from the active substance that presents no failures for any scenario.

Table B.9.4.4-9: Fish acute-term risk for BAS 750 F using the worst-case (tier 1) FOCUS STEP 3 PEC_{SW} values following application in spring and winter cereals

Test organism	RAC [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L] *
Spring cereals					
O. mykiss	5.32	D1	ditch	Multiple	1.703
			stream	Single	0.846
		D3	ditch	Single	0.948
		D4	pond	Multiple	0.089
			stream	Single	0.775
		D5	pond	Multiple	0.048
			stream	Single	0.796
		R4	stream	Single	0.627
Winter cereals					
O. mykiss	5.32	D1	ditch	Multiple	1.558

Test organism	RAC [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L] *
			stream	Single	0.841
		D2	ditch	Multiple	2.456
			stream	Multiple	1.533
		D3	ditch	Single	0.948
		D4	pond	Multiple	0.075
			stream	Single	0.791
		D5	Pond	Multiple	0.052
			stream	Single	0.756
		D6	ditch	Single	0.952
		R1	pond	Multiple	0.153
			stream	Multiple	0.684
		R3	stream	Single	0.877
		R4	stream	Single	0.627

* Tier 1 (standard parametrization)

STEP 4

There were failures in the risk assessments at STEP 3, i.e. there was an exceedance of the RAC for the acute risk to fish from the formulated product for the ditch and stream scenario as well as failures for the long-term risk to aquatic invertebrates for the D1 ditch (spring use) and D2 ditch (winter use); as a result further refinement of the exposure values is necessary at STEP 4.

Presented in Table B.9.4.4-10 is the risk assessment for the acute risk to fish from the formulated product.

Table B.9.4.4-10: Acute risk assessment for *O. mykiss* exposed to BAS 750 01 F expressed as BAS 750 F in f.p. using the spray drift only values assuming a 5m no spray buffer zone PEC_{SW} values following application in spring and winter cereals

Test organism	Application rate BAS 750 f (g/ha)	RAC [µg/L]	Scenario	$PEC_{SW, ini}$ [µg/L]
O. mykiss	150	0.524	Ditch	0.2612
			Pond	0.0284
			Stream	0.2612

PECs shown in **bold** are greater than the RAC

Table B.9.4.4-10 indicates an acceptable risk for all ditch, pond and stream scenarios at STEP 4 so no further consideration is required.

Presented in Table B.9.4.4-7 is the risk assessment for the long-term/chronic risk to *Daphnia magna* from the active substance.

Table B.9.4.4-11: Long-term risk assessment for *D. magna* exposed to BAS 750 F using the FOCUS STEP 4 (5m buffer zone) PEC_{SW} values following application in winter cereals

Test organism	Endpoint [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L]
Spring cereals					
D. magna	1.61	D1	ditch	Multiple	1.237
Winter cereals					
D. magna	1.61	D2	ditch	Multiple	**2.456**

Besides the standard aquatic invertebrate species *D. magna*, three additional chronic toxicity studies using aquatic invertebrate were submitted. These studies were considered reliable and used the active substance BAS 750 F and *A. bahia*, *D. pulex* and *D. longispina;* (see Table B.9.4-1). The endpoint from the *A. bahia* study is an unbounded NOEC, but is the most sensitive. By including the endpoint within the geometric mean calculation, the endpoint is more conservative than if the endpoint was not included.

Table B.9.4.4-12 Calculation of the geometric mean for the chronic endpoint of aquatic invertebrates based upon available chronic data

Chronic toxicity to aquatic invertebrates				
Daphnia magna	BAS 750 F	21d EC_{10}	16.1 µg a.s./L	III CA B.9.4.5/1, Janson, 2014a
Daphnia longispina		21d EC_{10}	56.4 µg a.s./L	III CA B.9.4.5/2, Janson, 2014b
Daphnia pulex		21d EC_{10}	56.7 µg a.s./L	III CA B.9.4.5/3, Janson, 2015a
Americamysis bahia		28d NOEC	≥ 13.2 µg a.s./L[1]	III CA B.9.4.5/4, Dinehart, 2016a
Geometric mean			28.7 µg a.s./L	

[1] for the purposes of generating the geometric mean, the NOEC has been taken as 0.0132 µg a.s./L

In section 2.1.4.1 of EFSA Aquatic GD (EFSA Journal 2013;11(7):3290) it is stated that the geometric mean approach may be used to refine the toxicity endpoint when toxicity data for a limited number of additional test species are available. EFSA states preconditions (see Sections 8.3.2 and 8.3.3) that should be considered prior to use of a geometric mean; these are detailed below.:

- *similar endpoints:*
  NOEC values are all derived from chronic studies over similar test durations (*i.e*. 21-28 days) and are based on the same effect parameter, *i.e.* effects on reproduction (number of offspring/parent)
- *species of the same taxonomic group:* aquatic crustaceans (aquatic insects, e.g. *Chironomus* proved to be less sensitive)
- *available data exceed the first tier data requirements:*
  Additional studies on *D. pulex*, *D. longispina* and *A. bahia*
- *most sensitive species should not be more than a factor of 100 below the geometric mean:*
  The study with *D. magna* provides the lowest endpoint, which is a factor of 2 below the geometric mean of all four chronic species.
- *Less than 8 species tested:*
  Four different species

- *If the lowest toxicity value is higher than the Geomean-RAC value, it is acceptable to use the Geometric mean approach:*
  The *Americamysis bahia* NOEC of 13.2 µg a.s./L is ~4 times higher than the $RAC_{geomean}$ of 2.87 µg a.s./L

On the basis of the above, it is deemed the four studies are reliable and can be used to determine a the **Geomean-$RAC_{chronic}$ of 28.7 µg a.s./L**. This RAC will be used with FOCUS STEP 3 and relevant STEP 4 $PEC_{SW, max}$ values and the resulting assessment is presented in Table B.9.4.4-13. Only the D1 and D2 scenarios are refined as these did not pass the standard risk assessment that used just the *Daphnia magna* endpoint. For the failing STEP 3 scenarios, the geomean RAC has been used to perform the risk assessment for both multiple and single applications.

Table B.9.4.4-13: Long-term risk for aquatic invertebrates exposed to BAS 750 F using the Geomean-$RAC_{chronic}$ with unresolved worst-case (tier 1) FOCUS STEP 3 PEC_{SW} values following application in spring and winter cereals, and multiple application FOCUS STEP 4 (5m buffer zone) PEC_{SW} values following application in winter cereals

Geomean-$RAC_{chronic}$ [µg/L]	FOCUS Scenarios		Application	$PEC_{SW, max}$ [µg/L] *
STEP 3				
Spring cereals				
2.87	D1	ditch	Multiple	1.703
Winter Cereals				
2.87	D2	ditch	Multiple	2.456
STEP 4				
Winter cereals				
2.87	D2	ditch	Multiple	2.456

PECs shown in **bold** are greater than the RAC
* Tier 1 (standard parametrization)

The $PEC_{SW, max}$ for BAS 750 F does not exceed the RAC at STEP 4 for any scenario.

Risk to sediment dwelling organisms

In addition to surface water, the risk of BAS 750 F to sediment dwelling organisms must be considered in a tiered approach. In order to assess the risk arising from sediment exposure, RACs were calculated using the 28 d NOEC values for BAS 750 F derived from chronic spiked-sediment studies with *C. riparius* and the worst-case FOCUS STEP 1 and 2 $PEC_{sed, max}$ values.

Table B.9.4.4-14: Long-term risk assessment for *C. riparius* exposed to BAS 750 F using the FOCUS STEP 1 and STEP 2 $PEC_{sed, max}$ values (sediment exposure)

Test substance	Test organism	RAC [µg/kg dry sediment]	FOCUS STEP	Applications	Scenario	$PEC_{sed, max}$ [µg/kg dry sediment]
Winter cereals*						
BAS 750 F	*C. riparius*	≥ 115.8	1	-	-	**630.572**
			2	Single	North	56.776
					South	105.191
				Multiple	North	108.973
					South	**203.511**

PECs shown in **bold** are greater than the RAC

* PEC values have been calculated for application in winter cereals, also covering application in spring cereals as the PEC values are identical for both applications

The $PEC_{sed, max}$ for BAS 750 F exceeds the RAC based on FOCUS STEP 2 calculation for multiple applications in southern Europe. Therefore, additional calculations considering worst-case STEP 3 PEC_{sed} values are presented in Table B.9.4.4-15.

Table B.9.4.4-15: Long-term risk assessment for *C. riparius* exposed to BAS 750 F using the worst-case (tier 1) FOCUS STEP 3 PEC_{sed} values following application in spring and winter cereals

Test organism	RAC [µg/kg dry sediment]	FOCUS Scenarios		Application	$PEC_{sed, max}$ [µg/kg dry sediment] *
Spring cereals					
C. riparius	≥ 115.8	D1	ditch	Multiple	21.900
			stream	Multiple	11.580
		D3	ditch	Multiple	0.769
		D4	pond	Multiple	0.814
			stream	Multiple	0.299
		D5	pond	Multiple	0.484
			stream	Multiple	0.054
		R4	stream	Multiple	4.221
Winter cereals					
C. riparius	≥ 115.8	D1	ditch	Multiple	20.660
			stream	Multiple	10.670
		D2	ditch	Multiple	21.990
			stream	Multiple	12.120
		D3	ditch	Multiple	0.763
		D4	pond	Multiple	0.731
			stream	Multiple	0.244
		D5	pond	Multiple	0.510

Test organism	RAC [µg/kg dry sediment]	FOCUS Scenarios		Application	$PEC_{sed, max}$ [µg/kg dry sediment] *
			stream	Multiple	0.062
		D6	ditch	Multiple	1.406
		R1	pond	Multiple	2.040
			stream	Multiple	3.162
		R3	stream	Multiple	2.800
		R4	stream	Multiple	4.127

* Tier 1 (standard parametrization)

The $PEC_{sed, max}$ for BAS 750 F are below the RAC based on FOCUS STEP 3 calculations, indicating low chronic risk to sediment dwelling aquatic insects *via* sediment exposure following the proposed uses of BAS 750 01 F close to surface waters.

B.9.4.5 Risk to aquatic organisms from metabolites

The risk posed by metabolites must be considered for both surface water and sediment compartments according to the results of fate and behaviour studies. For both the surface water and sediment compartments, M750F001, M750F005, M750F006, M750F007, M750F008 and M750F003 require consideration.

A summary of the fate and behaviour of metabolites is presented in III CP B.8.4. Both M750F001 and M750F003 were considered major metabolites based on the water/sediment study (III CA 7.2.2.3/1). Metabolites M750F005, M750F006, M750F007 and M750F008 were concluded to be 'major metabolites' for surface water based upon an aqueous photolysis study (III CA **Error! Reference source not found.**). In addition, due to the high estimated K_{OC} values, it was concluded that these metabolites could dissipate into the sediment if formed in the water column. The assessment of the photolysis metabolites is based on worst case assumptions.

A summary of the data available for each metabolite and the active substance is presented in Table B.9.4.5-1. It is noted that fish endpoints for M750F003, M750F005 and M750F008 are derived from QSAR data. As no data is available for sediment dwelling organisms, it is proposed to use the endpoint of the parent compound tested with C. riparius. Additional safety factors are not considered necessary as none of the other aquatic test organisms (including aquatic invertebrates) indicated higher toxicity.

Table B.9.4.5-1 Toxicity endpoints and physical chemical parameters for aquatic organisms and metabolites of BAS 750 F

Substance	Aquatic group endpoint (mg/L or mg/kg)				Log K_{OW}	Water solubility[1] (mg/L)	Estimated K_{OC}[2]
	Fish	*Daphnia*	Algae	Sediment dweller			
BAS 750 F	0.532	0.944	1.352[3]	≥1.158	3.4	0.81	3456
M750F001	498	>100	22.5	≥1.158[5]	-1	700000	89
M750F003	>100[4]	>100	>100	≥1.944	0.41	2460	597.6
M750F005	11.3[4]	>8.58	>8.57	≥1.158[5]	1.69	11.3	7863
M750F006	6.2	4.42	1.42	≥1.158[5]	2.73	11.2	4919
M750F007	>7.2	>10	>10	≥1.158[5]	0.90	72.7	3938
M750F008	>1.96[4]	>8.07	4.08	≥1.158[5]	1.76	1.96	17240

[1] Presented in III CA B.2.5

[2] Presented in III CA B.8.1.2.2

[3] *P. subcapitata* has been used as the species for the active substance to enable a like for like comparison with the metabolites that were all performed on *P. subcapitata*. As all the metabolite endpoints are less toxic than the BAS 750 F. *P. subcapitata* endpoint, the critical algae endpoint, *S. costatum* with an E_rC_{50} of 0.679 mg/L, will also be more toxic than the metabolites.

[4] Based upon QSAR data, presented in III CA B.9.12

[5] Same as the active substance, toxicity is not expected to be greater than the active from supporting data

Risk assessment for metabolites of BAS 750 F

Based upon the supporting data, it can be concluded that the toxicity of the M750F003, M750F005 and M750F008 to fish is not expected to be greater than that of the active substance and endpoints from the QSARs are considered suitable for use in the risk assessment. Five metabolites show no toxicity up to water solubility and all show lower toxicity than the active substance for aquatic invertebrates (Table B.9.4-22). Table B.9.4.5-2 presents the RACs for each relevant metabolite.

Table B.9.4.5-2 RACs for aquatic organisms and metabolites of BAS 750 F

Substance	Aquatic group endpoint (mg/L or mg/kg)				RAC (µg/L or µg/kg))			
	Fish	*Daphnia*	Algae	Sediment dweller	Fish	*Daphnia*	Algae	Sediment dweller
Trigger value	100	100	10	10	--	--	--	--
BAS 750 F	0.532	0.944	1.352	≥1.158	**5.32**	9.44	135.2	**≥115.8**
M750F001[4]	498	>100	22.5	≥1.158[5]	4980	>1000[4]	2250	**≥115.8**[5]
M750F003	>100[3]	>100	>100	≥1.944	**>1000**[3]	>1000	>10000	**≥194.4**
M750F005	11.3[3]	>8.58	>8.57	≥1.158[5]	113[3]	**>85.8**	>857	**≥115.8**[5]
M750F006	6.2	4.42	1.42	≥1.158[5]	62	**44.2**	142	**≥115.8**[5]
M750F007	>7.2	>10	>10	≥1.158[5]	**>72**	>100	>1000	**≥115.8**[5]
M750F008	>1.96[3]	>8.07	4.08	≥1.158[5]	**>19.6**[3]	>80.7	408	**≥115.8**[5]

Bold indicates the critical RAC for surface water and sediment compartments

[1] Presented in III CA B.2.5

[2] Presented in III CA B.8.1.2.2

[3] Based upon QSAR data, presented in III CA B.9.12

[4] The chronic fish RAC is more sensitive than all the acute RACs and is considered to be the critical RAC

[5] Same as the active substance, toxicity is not expected to be greater than the active from supporting data

It is noted that in addition to the RACs presented in Table B.9.4.5-2 above, M750F001 also has a chronic fish endpoint available. For a 28d NOEC of 3.2 mg/L for *Oncorhynchus mykiss* and an assessment factor of 10, the RAC of 320µg/L is the critical RAC for M750F001 and will be used in the risk assessments. For all the other metabolites, the critical RACs in **bold** in Table B.9.4.5-2 have been considered for risk in the surface water and sediment compartments below.

Surface water

Table B.9.4.5-3: Risk assessment for fish and aquatic invertebrates exposed to the major metabolites of BAS 750 F using the worst-case FOCUS STEP 1 $PEC_{SW, max}$ values

Test substance	Test organism	RAC [µg/L]	FOCUS Step	$PEC_{SW, max}$ [µg/L]
Winter cereals*				
1,2,4-triazole (M750F001)	*O. mykiss*	320[2]	1	3.209
M750F003	*O. mykiss*	>1000[1]	1	4.308
M750F005	*D. magna*	>85.8	1	3.521
M750F006	*D. magna*	44.2	1	0.646
M750F007	*O. mykiss*	>72	1	1.056
M750F008	*O. mykiss*	>19.6[1]	1	0.09

* PEC values have been calculated for application in winter cereals, also covering application in spring cereals.
[1] Based upon QSAR data, presented in III CA B.9.12

[2] RAC based upon chronic endpoint

As all the $PEC_{SW, max}$ for all the metabolites are smaller than the critical RAC, the risk can be considered low for all metabolites. Furthermore, as the critical RAC passed first tier STEP 1, no further consideration of the risk from metabolites to surface dwelling aquatic organisms is required as these groups have RACs greater than the critical RAC and therefore are covered by the acceptable risk presented above and require no further consideration.

Presented in Table B.9.4.5-4 is the risk assessment using FOCUS STEP 1 PECs for the sediment dwelling organisms from the metabolites 1,2,4-triazole (M750F001), M750F003, M750F005, M750F006, M750F007 and M750F008.

Table B.9.4.5-4: Risk assessment for sediment dwellers exposed to the major metabolites of BAS 750 F using the worst-case FOCUS STEP 1 $PEC_{sed, max}$ values for <u>spring and winter cereals</u>

Test substance	Test organism	RAC [µg/L]	FOCUS Step	$PEC_{sed, max}$ [µg/L]
Winter cereals*				
1,2,4-triazole (M750F001)	*Chironomus riparius*	≥115.8	1	2.847
M750F003	*Chironomus riparius*	≥115.8[1]	1	25.278
M750F005	*Chironomus riparius*	≥194.4	1	**215.874**
M750F006	*Chironomus riparius*	≥115.8[1]	1	**183.503**
M750F007	*Chironomus riparius*	≥115.8[1]	1	**240.821**
M750F008	*Chironomus riparius*	≥115.8[1]	1	48.196

* PEC values have been calculated for application in winter cereals, also covering application in spring cereals.
[1] Same as the active substance, toxicity is not expected to be greater than the active from supporting data

Presented in Table B.9.4.5-5 is the risk assessment using FOCUS STEP 2 PECs for the sediment dwelling organisms from the metabolites, M750F005, M750F006 and M750F007.

Table B.9.4.5-5: Risk assessment for sediment dwellers exposed to the major metabolites of BAS 750 F using the worst-case FOCUS STEP 2 $PEC_{sed, max}$ values for spring and winter cereals

Test substance	Test organism	RAC [µg/L]	FOCUS Step	$PEC_{sed, max}$ [µg/L]	
				Single application	Multiple application
Winter cereals*					
M750F005	*Chironomus riparius*	≥194.4	2	36.040	69.812
M750F006	*Chironomus riparius*	≥115.8[5]	2	30.635	59.344
M750F007	*Chironomus riparius*	≥115.8[5]	2	40.205	77.880

* PEC values have been calculated for application in winter cereals, also covering application in spring cereals.
1 Same as the active substance, toxicity is not expected to be greater than the active from supporting data

As all the $PEC_{SW, max}$ for all the metabolites are smaller than the critical RAC at either STEP 1 or STEP 2, the risk to sediment dwellers can be considered low for all metabolites.

B.9.4.6 Risk to aquatic organisms from the formulated product

The risk to aquatic organisms from the formulated product has been assessed within the assessment of the active substance using endpoints for the formulated product expressed in terms of active substance concentration. See B.9.4.4 for the full risk assessment.

B.9.4.7 Amphibian tadpoles

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active substance and the plant protection products, respectively), the risk to amphibians shall be addressed. In the EU there is no guidance or validated regulatory protocols yet available either on the type of regulatory testing necessary or how to conduct a risk assessment for amphibians.

B.9.4.8 Groundwater

Concentrations of BAS 750 F and relevant metabolites occurring in groundwater following the representative use on spring and winter cereals are modelled in Section B.8.3. PECgw values calculated were based on using the models FOCUS-PEARL 4.4.4, FOCUS-PELMO 5.5.3 and FOCUS-MACRO5.5.4. Modelling was performed for the parent compound (BAS 750 F) and the soil metabolite 1,2,4,- triazole. Exposure of aquatic organisms may occur where groundwater becomes surface water. The 80th percentile annual average PECgw concentrations at 1m soil depth were calculated as <0.1µg/L for both BAS 750 F and the soil metabolite 1,2,4- triazole when applied to spring and winter cereals. Therefore, it is considered that risk of groundwater contamination at >0.1µg.L from the proposed use of BAS 750 1 F is unlikely and therefore a low risk to aquatic organisms from BAS 750 F and its metabolites exposed via groundwater was concluded.

B.9.4.9 Bioaccumulation

The log K_{OW} value of 3.4 for BAS 750 F is greater than the log K_{OW} trigger value of 3. An experimental bioconcentration study in fish is available to consider bioaccumulation further. In the experimental study, whole fish BCF values for BAS 750 F of 385 were less than 500 indicating a low potential for bioaccumulation. Additionally, rapid depuration of BAS 750 F was observed with a depuration $t1/2_g$ of 0.60 days (based on total radioactivity). These data indicate BAS 750 F rapidly accumulates and is eliminated in fish.

Table B.9.4.9-1: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water Calculation based on solubility in water and *n*-octanol	Results determined at 20 °C applying the HPLC method. pH 4*: log K_{OW} = 3.4 pH 7: log K_{OW} = 3.4 pH 7*: log K_{OW} = 3.3 pH 9*: log K_{OW} = 3.4	Valid Log K_{OW} is not pH dependent	Wilbrand, 2013c
Experimental aquatic BCF OECD Guideline 305, GLP	Steady state whole fish lipid-normalized kinetic BCF_{KLg}: 385 $t1/2_g$ (growth-corrected depuration half-life): 0.60 day	Flow through, 14 days exposure, 7 days depuration	[redacted] 2015c

* buffered

Using worst-case PEC_{SW} BAS 750 F values (max, FOCUS STEP 1) an acceptable risk to both fish-eating birds and fish-eating mammals from BAS 750 F was demonstrated (Tables B.9.1.1.2-3 and B.9.1.2.2-4).

B.9.4.10 Overall Conclusion – risk to aquatic organisms

The proposed use of BAS 750 F on spring and winter cereals results in an acceptable risk to aquatic organisms as the risk is resolved in all aquatic groups up to STEP 4 for all scenarios, and therefore representing the necessary safe scenarios (Table B.2.10-1).

Table B.9.4.10-1: Summary of risk posed to aquatic organisms from the active substance

Test substance	Test group	Test species	RAC (µg/L or µg/kg)	Maximum STEP assessed	Failing scenarios at STEP 4
BAS 750 F	Acute fish	*O. mykiss*	5.32	STEP 3	-
	Chronic fish	*D. rerio*	2.7	STEP 3	-
	Acute aquatic invertebrates	*D. magna*	9.44	STEP 2	-
	Chronic aquatic invertebrates	*D. magna*	1	STEP 4	none
	Algae	*S. costatum*	67.9	STEP 1	-
	Aquatic plants	*L. gibba*	>201.7	STEP 1	-
	Sediment dwelling organisms	*C. riparius*	≥ 115.8	STEP 3	-
BAS 750 01 F (expressed as a.s. in f.p.)[1]	Acute fish	*O. mykiss*	0.524	STEP 4	none
	Acute aquatic invertebrates	*D. magna*	1.813	STEP 3	-
	Algae	*P. subcapitata*	8.51	STEP 2	-

[1]Calculated based upon a density of 0.993g/cm^3

As the assessment for the formulated product has already been covered in the active assessment by using endpoints for BAS 750 01 F expressed as BAS 750 F in f.p., no further consideration of the risk posed by the formulated product is required. It is concluded that no BAS 750 F groundwater or bioaccumulatory risks to aquatic life are identified.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

Report: B.9.5.1/1
Franke M., 2015a
Acute toxicity of BAS 750 01 F to the honeybee *Apis mellifera* L. under laboratory conditions
2014/1242741
Guidelines: OECD 213 (1998)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006;
Content of a.s.: BAS-750 F 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: *Apis mellifera* L. subspecies *iberica* G. (honeybee), young adult worker bees (3 - 5 weeks old) derived from a healthy and queen-right colony; source: beekeeper J. Cordero, Cazalla, Spain; randomly collected in the morning prior to use from the top of the bee hive.

Test design: In a 48 hour test, young adults of *Apis mellifera* were exposed orally to BAS 750 01 F via treated food (50% w/v aqueous sucrose solution). The initial feeds, containing the test doses, were all consumed within 2 hours, and replaced with feeding tubes containing untreated 50 % sucrose solution. In total, 3 treatment groups were set up (5 concentrations of the test substance, 1 untreated control and 4 concentrations of the reference item) with 3 replicates per concentration and 10 bees per replicate. Assessment of bee mortality and behavioural effects were done after 4, 24 and 48 hours.

Endpoints: Mortality (LD_{50}), behavioural impairments.

Reference item: Dimethoate EC 400 (dimethoate: 400.9 g/L analysed).

Test concentrations: BAS 750 01 F: 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee in an aqueous sucrose solution (50% w/v); resulting in an actual uptake of 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee (equivalent to 124.1, 248.3, 496.5, 993.0 and 1986.0 µg BAS 750 01 F/bee). The test doses were prepared by step-wise serial dilution of a 99.3 ug/L stock solution of the formulation in 50 % w/v aqueous sucrose, such that each replicate received the corresponding dose in 236.4 mg solution.
Control - 50% w/v sucrose solution.

Reference item: 0.086, 0.122, 0.175 and 0.250 µg dimethoate/bee, resulting in an uptake of 0.086, 0.122, 0.175 and 0.250 µg dimethoate/bee.

Test conditions: Temperature: 23.8°C – 26.9°C; relative humidity: 50% - 69%, photoperiod: 24 h darkness; food: 50% w/v sucrose solution. The bees were kept in disposable cardboard cages with a glass front and ventilation holes in the bottom (95 x 50 x 65 mm).

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$), Moving Averages Computation and Probit analysis using linear maximum likelihood regression for calculation of the LD_{50} values of the test substance and reference item. Statistical software used was ToxRat Professional 3.0 beta (2014).

II. RESULTS AND DISCUSSION

The results for mortality are summarised in the table below. At 4 hours post-treatment, all test groups fed with the test substance exhibited moribundity and impaired locomotion, with greater incidence in the higher test doses (>124.1 µg BAS 750 F 01/bee). At 24 hours and 48 hours, surviving bees at the test doses 1986, 993, and 496.5 µg BAS 750 F 01/bee displayed moribundity and impaired locomotion. No effects on behaviour were observed in the lower two dose rates at both of these time points. The LD_{50} value (48 h) was determined to be 409.6 µg BAS 750 01 F/bee, which is equivalent to 41.2 µg a.s./bee. The results are summarised in Table B.9.5.1/1-1.

Table B.9.5.1/1-1: Toxicity of BAS 750 01 F to *Apis mellifera* (honeybee) in an oral toxicity test

Treatment [µg a.s./bee]	**Actual Uptake of a.s. [µg a.s./bee]**	**Actual Uptake of test substance [µg BAS 750 01 F/bee]**	**Mean mortality [%]**	
			24 h	**48 h**
Sucrose control	--	--	0.0	0.0
12.5	12.5	124.1	0.0	0.0
25.0	25.0	248.3	3.3	6.7
50.0	50.0	496.5	66.7 *	66.7 *
100.0	100.0	993.0	93.3 *	93.3 *
200.0	200.0	1986.0	83.3 *	83.3 *
Reference Item – Dimethoate EC 400 [µg a.s./bee]	**Actual Uptake of a.s. [µg a.s./bee]**	**Actual Uptake of test substance [µg Dimethoate EC 400/bee]**	**24 h**	**48 h**
0.086	0.086	0.228	26.7*	36.7*
0.122	0.122	0.326	50.0*	56.7*
0.175	0.175	0.466	83.3*	86.7*
0.250	0.250	0.666	93.3*	96.7*
	Endpoint [µg/bee]			
	Based on a.s. in BAS 750 01 F	**Based on product BAS 750 01 F**		

LD_{50} (48 h) (95% confidence limits)	41.2 (33.5 – 50.7)	409.6 (333.1 – 503.8)

* Statistically significantly different compared to the control (Fisher's Exact Binominal test, one-sided greater, $\alpha = 0.05$).

The LD_{50} value (24 h) for the reference item was determined to be 0.117 µg dimethoate/bee (95% confidence limits: 0.103 - 0.134 µg dimethoate/bee), based on consumption.

Validity Criteria
The study meets the validity criteria specified in OECD 213:

- Average control mortality did not exceed 10 % (being 0 %)
- LD_{50} of the reference item (0.117 µg dimethoate/bee) falls within the range described in guidance (0.1-0.35 µg a.s./bee)

III. CONCLUSION

In an acute oral toxicity study with BAS 750 01 F on honeybees, the LD_{50} value (48 h) was determined to be 409.6 µg BAS 750 01 F/bee, which is equivalent to 41.2 µg a.s./bee.

RMS Comments
The study was carried out according to the principles of GLP and follows OECD guideline 213 with no deviations noted. The study meets all of the validity criteria specified in the guidance. In all replicates at each test dose, all of the sucrose solution treated with the test substance was consumed within 2 hours. This supports the use of nominal doses in calculating the endpoints. It was noted that the endpoint expressed in terms of the active substance was calculated by multiplying the formulation endpoint by 0.993 (reflecting the density of the formulation). The RMS has recalculated this value using the following assumptions: BAS 750 01 F contains 98.9 g/L BAS 750 F, and the density of the formulation is 0.993 g/cm^3. Therefore:

(98.9 / 993) = 0.0996 BAS 750 F (w/w)

LD_{50} = 409.6 µg f.p./bee

(409.6 x 0.0996) = 40.8 µg a.s./bee.

This is a minor deviation from the endpoint calculated in the study (this being 41.2 µg a.s/bee). Therefore the RMS does not consider it necessary to alter this endpoint for the purposes of the risk assessment.

The agreed endpoint considered suitable for use in the risk assessment is:

48 h LD_{50} = 409.6 µg BAS 750 01 F/bee (41.2 µg a.s./bee)

Report: B.9.5.1/2
Franke M., 2015a
Acute toxicity of BAS 750 01 F to the honeybee *Apis mellifera* L. under laboratory conditions
2014/1242741
Guidelines: OECD 214 (1998)

GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006;
Content of a.s.: BAS 750 F 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: *Apis mellifera* L. subspecies *iberica* G. (honeybee), young adult worker bees (3 - 5 weeks old) derived from a healthy and queen-right colony; source: beekeeper J. Cordero, Cazalla, Spain; randomly collected in the morning prior to use from the top of the bee hive.

Test design: In a 96-hour test, young adults of *Apis mellifera* were exposed to 5 concentrations of BAS 750 01 F in an appropriate carrier (Tween 80) placed on the dorsal bee thorax. In total, 4 treatment groups were set up (5 concentrations of the test substance, water control, tween control and 4 concentrations of the reference item) with 3 replicates per concentration and 10 bees per replicate. Assessment of bee mortality and behavioural effects were done after 4, 24, 48, 72 and 96 hours.

Endpoint: Mortality (LD_{50}), behavioural impairments.

Reference item: Dimethoate EC 400 (dimethoate: 400.9 g/L analysed).

Test concentrations: BAS 750 01 F: 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee (equivalent to 124.1, 248.3, 496.5, 993.0 and 1986.0 µg BAS 750 01 F/bee), deionized water control, tween control (1.0% v/v Tween 80 solution), reference item: 0.105, 0.141, 0.187, 0.250 µg dimethoate/bee. Bees were anaesthetised with CO_2 for approximately 20 seconds prior to application of the treatment solutions. The controls and test substance groups received 4 µL doses whilst the reference item group received 2 µL doses. The test substance doses were prepared by serial dilution of a stock solution of the highest concentration used (1986 µg f.p./bee).

Test conditions: Temperature: 23.8°C - 26.9°C; relative humidity: 50% - 69%, photoperiod: 24 h darkness; food: 50% w/v sucrose solution. The bees were kept in disposable cardboard cages with a glass front and ventilation holes in the bottom (95 x 50 x 65 mm).

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$), Probit analysis using linear maximum likelihood regression and Moving Averages Computation for calculation of the LD_{50} values of the test substance and reference item. Statistical calculations were carried out using ToxRat Professional 3.0 beta (2014).

II. RESULTS AND DISCUSSION

Due to a ≥10 % increase of mortality between the 24 hour and 48 hour assessment, the contact toxicity test was extended up to 96 hours. The results for mortality and behavioural effects are summarised in the table below.

Table B.9.5.1/2: Toxicity of BAS 750 01 F to *Apis mellifera* in a contact toxicity test

Treatment	Treatment	24 h		48 h		72 h		96 h	
	[µg BAS 750 01 F/bee]	Mean mortality [%]	Bees with abnormal behaviour (%)	Mean mortality [%]	Bees with abnormal behaviour (%)	Mean mortality [%]	Bees with abnormal behaviour (%)	Mean mortality [%]	Bees with abnormal behaviour (%)
Water control	--	0	0	0	0	0	0	0	0
Tween control	--	0	0	0	0	0	0	0	0
12.5	124.1	0	0	0	0	3.3	3.3	16.7 *	3.3
25	248.3	13.3	3 3	13.3	3.3	16.7	3.3	20.0 *	13.3
50	496.5	80.0 *	6.6	80.0 *	6.6	80.0 *	6.6	83.3 *	6.6
100	993	73.3 *	26.6	90.0 *	10	96.7 *	3.3	100.0 *	n/a
200	1986	96.7 *	3 3	100.0 *	n/a	100.0 *	n/a	100.0 *	n/a
Reference Item (µg a.s./bee)									
0.105		10	3 3	13.3	3.3				
0.141		26.7*	10	36.7*	10				
0.187		80.0*	0	80.0*	10				
0.25		80.0*	3 3	83.3*	13.3				
	Endpoint [µg/bee]								
	Based on a.s. content of BAS 750 01 F				**Based on product BAS 750 01 F**				
LD_{50} (48 h) (95% confidence limits)	40.6 (27.9 – 58.9)				403.0 (277.5 – 585.3)				
LD_{50} (96 h) (95% confidence limits)	29.8 (18.3 – 48.6)				296.4 (181.9 – 483.0)				

* Statistically significantly different compared to the tween control (Fisher's Exact Binominal Test, one-sided greater, $\alpha = 0.05$).

The LD_{50} value (24 h) for the reference item was 0.161 µg dimethoate/bee (95% confidence limits: 0.137 - 0.188 µg dimethoate/bee) in the contact toxicity test.

Validity Criteria
The study meets the validity criteria specified in OECD 214:

- Average control mortality did not exceed 10 % (being 0 % in both controls)
- LD_{50} of the reference item (0.161 µg dimethoate/bee) falls within the range described in guidance (0.1-0.30 µg a.s./bee)

III. CONCLUSION

In an acute contact toxicity study with BAS 750 01 F on honeybees, the LD_{50} value (48 h) was determined to be 403.0 µg BAS 750 01 F/bee (equivalent to 40.6 µg a.s./bee) and the LD_{50} (96 h) was 296.4 µg BAS 750 01 F/bee (equivalent to 29.8 µg a.s./bee).

RMS Comments
The study was carried out according to the principles of GLP and follows OECD guideline 214 with one minor deviation noted. This was the use of 4 and 2 µL topical applications of controls and test substance doses, and reference item doses (respectively). The study authors justified this with the statement: 'BioChem agrar experience has proven that higher volumes than 1 µL droplets (as recommended in the guideline) are suitable and no adverse effects on the outcome of the study are to be expected.' The study meets all of the validity criteria specified in guidance, and guidance also states that other dose volumes may be used if justified; therefore this deviation is not thought to have had a significant impact on the study results. As the study duration had to be extended to 96 h, the RMS considers the 96 hour endpoint to be the most suitable for risk assessment. It was noted that the endpoint expressed in terms of the active substance was calculated by multiplying the formulation endpoint by 0.993 (reflecting the density of the formulation). The RMS has recalculated this value using the following assumptions: BAS 750 01 F contains 98.9 g BAS 750 F/L, and the density of the formulation is 0.993 g/cm^3. Therefore:

(98.9 / 993) = 0.0996 BAS 750 F (w/w)

LD_{50} = 296.4 µg f.p./bee

(296.4 x 0.0996) = 29.5 µg a.s./bee.

This is a minor deviation from the endpoint calculated in the study (this being 29.8 µg a.s/bee). Therefore the RMS does not consider it necessary to alter this endpoint for the purposes of the risk assessment.

The agreed endpoint considered suitable for use in the risk assessment is:
96 h LD_{50} = 296.4 µg BAS 750 01 F/bee (29.8 µg a.s./bee)

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

Report: B.9.5.2/1
Fallowfield L., 2015a
A rate-response laboratory test to determine the effects of BAS 750 01 F on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae)

2014/1242742
Guidelines: Blümel *et al*. (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F, 98.9 g/L analysed (100.0 g/L nominal); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Predatory mite (*Typhlodromus pyri*), protonymphs (less than 24 h old); source: in-house culture.

Test design: Exposure of the mites to air-dried residues on treated glass plates (open method, as per guidance). The plates were formed from two microscope slide cover slips (22 x 40 mm each), laid side by side and fixed together with additional cover slips which were glued to top and bottom ends of the main cover slips. A 3x4 cm (12 cm^2) oblong of non-drying insect glue formed the boundary of each test arena. Seven treatment groups (five test substance rates, water treated control and reference item), with 5 replicates in the control and 3 replicates per treatment group, each containing 20 mites. Assessment of mortality was done 1 and 7 days after treatment (DAT). Mites were transferred to the arenas within 1.5 h of treatment application.

Endpoints: Mortality after exposure over 7 days.

Reference item: BAS 152 11 I (Perfekthion), a.s.: dimethoate: 400.9 g/L (analysed).

Test rates: Control (purified water), 187.5, 375, 750, 1500 and 3000 mL BAS 750 01 F/ha. A stock solution of the highest test rate was prepared by diluting 3 mL test substance to 200 mL with purified water. The lower test rates were prepared by diluting appropriate volumes of the stock solution to 100 mL with purified water. The reference item was applied at an application rate of 15 mL/ha (6 g a.s./ha). All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates with a calibrated laboratory spraying equipment and air dried afterwards. Calibration took place in advance of applications using purified water. Three pre-weighed plates were sprayed using purified water. The plates were then re-weighed and the rate of deposition determined. Once three consecutive applications had delivered a rate of 2 mg deposit/cm^2 ± 10 % (application rates ranged between 98.5 and 108 % of nominal), treatments were applied.

Test conditions: Temperature: 24 °C – 25 °C; relative humidity: 72% - 89%; photoperiod: 16 h light: 8 h dark; light intensity: 680 - 1200 lux; food: 1:1 v/v mixture of pollen from almond and apple.

Statistics: Stuck, drowned and missing mites were added to the number of dead mites to derive the overall 'mortality'. Mean percentage mortality after 7 days was calculated for individual treatments and then corrected for any losses in the control using Abbott's formula (1925). Fisher's Exact Test for mortality (α =

0.05) was used to compare the mortality in each test substance treatment with that in the control; Probit regression analysis for calculation of LR_{50}. Analysis carried out using SPSS, 2012.

II. RESULTS AND DISCUSSION

Corrected mortalities of 6.3%, 16.7%, 49.7%, 72.2% and 100% were observed after 7 days in the 187.5, 375, 750, 1500 and 3000 mL BAS 750 01 F/ha treatment rates , respectively. Statistically, all treatment rates of BAS 750 01 F differed significantly from the control, with the exception of the lowest rate 187.5 mL/ha (Fisher's Exact Test, $\alpha = 0.05$). The results are summarised in Table B.9.5.2/1-1. It was noted that in the 750 and 1500 mL treatments not all of the mites were mature at 7 DAT.

Table B.9.5.2/1-1: Effects of BAS 750 01 F on predatory mites (*Typhlodromus pyri*) under worst-case laboratory conditions

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]
Control	--	4.0	--
BAS 750 01 F	187.5	10.0	6.3
	375	20.0 *	16.7
	750	51.7 *	49.7
	1500	73.3 *	72.2
	3000	100 *	100
Endpoints [mL BAS 750 01 F/ha]			
LR_{50} (95% CL) [4]	769.1 (633.9 – 907.8)		

1) Application rate in 200 L water/ha.
2) Mortality after 7 days of exposure to BAS 750 01 F on glass surface.
3) Corrected mortality according to Abbott (1925).
4) Median lethal rate with 95% upper and lower confidence limits.
* Statistically significant differences compared to the control (Fisher's Exact Test, $\alpha = 0.05$).

The reference item caused a corrected mortality of 96.5% at 7 DAT.

Validity Criteria
The study meets the following validity criteria as specified in Blümel et al. (2000):

- Mean control mortality did not exceed 20 % on day 7 (being 4.0 %)
- Reference item mortality, at 15 ml/ha, was between 50 and 100 % (being 96.5 %)

III. CONCLUSION

In a worst-case laboratory study with BAS 750 01 F, the LR_{50} for *Typhlodromus pyri* was 769.1 mL BAS 750 01 F/ha (applied in 200 L water/ha).

RMS Comments
The study was carried out according to GLP and follows the guideline described by Blümel et al. (2000) with one deviation noted. The study did not extend to include a reproductive assay, in the treatment groups where mortality was < 50 % (375 and 187.5 mL/ha), however as an extended laboratory test that covers sublethal effects has been submitted, and the Hazard Quotient (HQ) risk assessment approach relies on mortality data only, this is not considered to be a significant deviation.

The agreed endpoint considered suitable for use in the risk assessment is:
7 day LR_{50} = 769.1 mL BAS 750 01 F/ha

Report:	B.9.5.2/2 Stevens J., 2015a A rate-response laboratory test to determine the effects of BAS 750 01 F on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) 2014/1242743
Guidelines:	Mead-Briggs M. *et al.* (2000)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 98.9 g/L analysed (100.0 g/L nominal); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez), adults (< 48 hours old); source: in-house culture.

Test design: Exposure of adult parasitoids was achieved via air-dried residues on treated glass plates, assembled as per guidance (Mead-Briggs *et al.*, 2000). 7 treatments (5 test substance rates, water treated control, reference item) with 4 replicates each were set up. Each replicate contained 10 wasps. Assessment of mortality was carried out 2, 24 and 48 h after test initiation.

Endpoints: Mortality after exposure over 48 h, including the determination of a LR_{50}.

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

Test rates: Control (purified water), 46.875, 93.75, 187.5, 375 and 750 mL BAS 750 01 F/ha. A stock solution of 3000 mL BAS 750 01 F/ha was prepared by diluting 1.5 mL to 100 mL with purified water. The test rates were prepared by diluting appropriate volumes of the stock solution to 100 mL with purified water. The reference item was applied at an application rate of 0.1 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates via laboratory spraying equipment and air dried afterwards. The spraying equipment was calibrated in advance of applications using purified water. Three pre-weighed plates were sprayed, then reweighed and the rate of deposition determined. Once three consecutive applications had delivered a rate of 2 mg deposit/cm^2 ± 10 % (application rates ranged between 94.5 and 100 % of nominal), treatments were applied.

Test conditions: Temperature: 21°C; relative humidity: 70% - 74%; photoperiod: 16 h light: 8 h dark; light intensity: 1203 lux. Food: 1: 3 *v/v* solution of honey in water.

Statistics: Percentage mortality was calculated, based on the numbers of moribund and dead insects combined. The data were also corrected for any control treatment deaths using Abbott's Formula (1925). The individual test-item treatment mortalities were compared with that of the control with Fisher's exact test ($\alpha = 0.05$), Probit analysis for calculation of LR_{50}. Statistical analysis was carried out using SPSS (2012).

II. RESULTS AND DISCUSSION

After 48 h a mortality of 7.5% was observed in the control. In the test substance treatments, mortality ranged between 20.0% and 100%. This resulted in corrected mortality rates between 13.5% and 100%. Statistically significant effects on mortality were determined in the 93.75, 187.5, 375 and 750 mL BAS 750 01 F/ha test substance treatment groups (Fisher's exact test, $\alpha = 0.05$). The results are summarised in Table B.9.5.2/2-2.

Table B.9.5.2/2-2: Effects of BAS 750 01 F on parasitoids (*Aphidius rhopalosiphi)* under worst-case laboratory conditions

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]
Control	--	7.5	--
BAS 750 01 F	46.875	20.0	13.5
	93.75	55.0 *	51.4
	187.5	82.5 *	81.1
	375	100 *	100
	750	100 *	100
Endpoints [mL BAS 750 01 F/ha]			
LR_{50} (95% CL) [4]	95.4 (74.2 – 116.0)		

1) Application rate in 200 L water/ha.
2) Mortality after 48 h of exposure to BAS 750 01 F on glass plates.
3) Corrected mortality according to Abbott (1925).
4) Median lethal rate with 95% confidence limits.
* Statistically significant differences compared to the control (Fisher's exact test, $\alpha = 0.05$).

The reference item caused a corrected mortality of 100% of exposed wasps after 48 h.

Validity Criteria:
The study met the following validity criteria specified in Mead-Briggs *et al*. (2000):

- The control mortality did not exceed 13 % (being 7.5 %)
- The reference item treatment of 0.1 ml/ha resulted in a mortality of 100 %, confirming the sensitivity of the test organism.

III. CONCLUSION

In a worst-case laboratory study with BAS 750 01 F, the LR_{50} for *Aphidius rhopalosiphi* was 95.4 mL BAS 750 01 F/ha (applied in 200 L water/ha).

RMS Comments
The study was conducted according to GLP and follows the guideline specified in Mead-Briggs et al., (2000). It was noted that the study did not extend to including a fecundity assay after the mortality assessment was complete. This would normally result in the study being declared invalid, as the

fecundity assessment forms one of the validity criteria. However, extended laboratory studies have been provided – please see below.

The agreed endpoint considered suitable for use in the risk assessment is:

48 hr LR_{50} = 95.4 mL BAS 750 01 F/ha

Report:	B.9.5.2/3 Fallowfield L., 2015b A rate-response extended laboratory test to determine the effects of BAS 750 01 F on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) 2015/1020207
Guidelines:	Blümel *et al.* (2000)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed), density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Predatory mite (*Typhlodromus pyri*), protonymphs (less than 24 h old); source: in-house culture of the test facility.

Test design: Exposure of the mites to air-dried residues on treated leaf (*P. vulgaris* var. The Prince) discs. Treated discs were laid on water saturated cotton wool-lined Petri dishes. A strip of 'Benchkote' served as a water source for the mites. A sticky gel barrier drawn around the edge of the leaf disc prevented the mites from dispersing, giving an arena size of approximately 12.5 cm^2. 7 treatment groups (5 test substance rates, purified water control and reference item), with 5 replicates for the control and 3 replicates for each test substance rate, each containing 20 mites, respectively. Assessments of mortality were conducted 1 and 7 days after treatment (DAT). Reproduction of surviving mites was assessed in the 3 highest concentrations tested (any eggs produced prior to 7 DAT were discarded). Recording of number of eggs produced per female for an additional 7 days, on days 9, 11 and 14 DAT.

Endpoints: Mortality after 7 days, including determination of an LR_{50} and effects on reproduction.

Reference item: BAS 152 11 I (a.s.: dimethoate: 400.0 g/L nominal, 400.9 g/L analysed).

Test rates: Control (purified water), and 187.5, 375.0, 750.0, 1500.0 and 3000.0 mL BAS 750 01 F/ha. A stock solution of the highest rate tested (3000 mL/ha) was prepared by diluting 3 mL test substance to 200 mL with purified water. The lower test rates were prepared by diluting appropriate quantities of the stock solution to 100 mL with purified water. The reference item was applied at an application rate of 37.5 mL/ha. All substances were applied in 200.0 L water/ha. The substances were sprayed onto leaf discs with a

calibrated laboratory track-sprayer and the residues then left to dry. Calibration took place in advance of applications using purified water. Three pre-weighed plates were sprayed, re-weighed, and the rate of deposition determined. Once three consecutive applications had delivered the correct deposition rate of 200 L/ha (2 mg/cm^2), the treatments were applied to the pre-cut leaf discs. The actual deposition rate varied between 98 and 104.5 % of nominal during calibration.

Test conditions: Temperature: 24.0 °C – 25.0 °C; relative humidity: 57.0% - 84.0%; photoperiod: 16 h light: 8 h dark; light intensity: 900-1600 lux; food: 1:1 (v/v) mixture of pollen from almond *(Prunus* sp. var. Butte*)* and apple *(Malus* sp. var. Red Delicious).

Statistics: Stuck, drowned or missing mites were added to the number of dead mites found in each treatment to derive overall mortality. The mean % mortality was calculated for individual treatments and corrected for any losses in the control using Abbott's formula (Abbott, 1925). Mortality in each treatment was compared with that in the control using Fisher's Exact Test ($\alpha = 0.05$). Reproduction data was checked for normality (Shapiro-Wilk, $\alpha = 0.05$), then based on this result, compared with the control reproduction data using the non-parametric Mann-Whitney U-test (two-sided, $\alpha = 0.05$). Probit regression analysis for calculation of LR_{50}.

II. RESULTS AND DISCUSSION

After 7 days, the mortality in the test substance treatments ranged from 1.7% to 28.3% in comparison to 6.0% in the control. Statistically significant differences compared to the control were determined at the two highest treatment rates of 1500.0 and 3000.0 mL BAS 750 01 F/ha (Fisher's Exact Test, $\alpha = 0.05$).

The LR_{50} was calculated to be > 3000.0 mL BAS 750 01 F/ha in 200.0 L water/ha.

The mean number of eggs produced per female was 6.9 in the control, compared with 7.2, 6.7, and 5.9 in the 750.0, 1500.0 and 3000.0 mL BAS 750 01 F/ha treatment rates, respectively.
No treatment rates had statistically significant adverse effects on reproduction, relative to control. (Mann-Whitney *U*-test, two-sided, $\alpha = 0.05$).

The results are summarised in Table B.9.5.2/3-1.

Table B.9.5.2/3-1: Effects of BAS 750 01 F on predatory mites (*Typhlodromus pyri*) under extended laboratory conditions

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Mean number eggs per female [4] (7-14 DAT)	Effects on reproduction [5] [%]
Control	--	6.0	--	6.9	--
BAS 750 01 F	187.5	1.7	-4.6	--	--
	375.0	6.7	0.7	--	--
	750.0	7.5	1.6	7.2	-4.3

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Mean number eggs per female [4] (7-14 DAT)	Effects on reproduction [5] [%]
	1500.0	20.0 *	14.9	6.7	3.0
	3000.0	28.3 *	23.8	5.9	14.4
Endpoints [mL BAS 750 01 F/ha]					
LR_{50} [6]	**> 3000.0**				

1) Application rate in in terms of BAS 750 01 F per 200 L water/ha.
2) Mortality after 7 days of exposure on treated leaf discs.
3) Corrected mortality according to Abbott (1925).
4) Results for reproduction in individual test-item treatments compared to the control.
5) Change in numbers of eggs per female, relative to control. A positive value indicates a decrease, relative to the control.
6) Median lethal rate.
* Statistically significant differences compared to the control (Fisher's Exact Test, $\alpha = 0.05$).

The reference item caused a corrected mortality of 100% after 7 days of exposure (DAT).

Validity Criteria
The study meets all the validity criteria specified by Blümel *et al.* (2000):

- Control mean mortality did not exceed 20 % (being 6 %)
- Control mean number of eggs/female was not less than 4 (being 6.9)
- Reference item mortality at the rate of 37.5 mL/ha was 100 %

III. CONCLUSION

In an extended laboratory study with BAS 750 01 F, the LR_{50} for *Typhlodromus pyri* was estimated to be > 3000.0 mL BAS 750 01 F/ha in 200 L water/ha. The test substance caused no unacceptable adverse effects on reproduction at treatment rates up to and including 3000.0 mL BAS 750 01 F/ha (applied in 200.0 L water/ha).

RMS Comments
The study was carried out according to GLP and follows the guideline specified by Blümel et al. (2000) with a number of deviations.
The study followed a modified version of the *T. pyri* first tier laboratory study and in doing so applied a higher rate of the reference item (37.5 mL/ha, highest recommended in Blümel *et al.*, 2000, is 15 mL/ha). This introduces uncertainty, both into the role of the reference item and into the sensitivity of the batch of *T. pyri* used. However, regarding the effects of the test substance, it clearly shows that BAS 750 01 F is of low toxicity.
Secondly, it was noted that all mites escaped from one replicate in the 750 mL/ha treatment – so the mortality and reproduction results for this treatment are based on 2 replicates. Thirdly, the study did not include fecundity assessments of the mites treated with the two lowest test rates (187.5 and 375.0 mL/ha). Guidance states that fecundity assessments are to be carried out for all groups where mortality during the initial 7 days after treatment does not exceed 50 %. As the mortality was less than 50 % in both these groups (being -4.6 and 0.7 % respectively), both should have undergone the fecundity assessment. However as there were no significant effects of the test substance on fecundity in the treatment groups that received the three highest test rates, it can be concluded that it is very unlikely that significant effects would be observed at the two lowest test rates. This is therefore not considered to be a significant deviation, and overall the study is considered acceptable for use in the risk assessment.

The agreed endpoints considered suitable for use in the risk assessment are:

- **7 day LR_{50} ≥3000 mL BAS 750 01 F /ha**
- **ER_{50} reproduction ≥3000 mL BAS 750 01 F /ha**

Report: B.9.5.2/4
Stevens J., 2015b
A rate-response extended laboratory test to determine the effects of BAS 750 01 F on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)
2014/1242744
Guidelines: Mead-Briggs M. *et al.* (2009)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Parasitic wasp *(Aphidius rhopalosiphi*); adults; age: less than 48 h; source: in-house culture, maintained on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*).

Test design: Adult female parasitoids were exposed to air-dried residues applied to treated barley seedlings (*Hordeum vulgare)*. The seedlings (approximately 10 per pot) were grown in 11 cm diameter pots, and were 8 days old with two leaves (BBCH 12) at the start of the study. The plants were approximately 10 cm in height at the start of the study. Approximately 70 minutes before product application the seedlings were sprayed with a 10 % w/v solution of fructose. Once treated with the product, the plants were enclosed within clear acrylic cylinders (8 x 20 cm diameter x height), topped with a mesh of nylon netting. The study included 7 treatment groups (5 test substance treatments, purified water control, reference item) with 6 replicates per treatment, each containing 5 female wasps. Assessment of the repellence of wasps from the freshly treated plants was made during the first 3 h after their release and at 24 and 48 h after treatment. The mortality was assessed 2, 24 and 48 hours after test initiation. At 48 h, surviving wasps (n = 15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi* and *Metopolophium dirhodum*. Assessments were made for the control and the 3 highest treatment rates of test substance that resulted in ≤ 60% corrected mortality. The adult wasps were removed after 24 h and the aphid-infested plants left for a further 10 days before the numbers of aphid mummies that had developed was assessed.

Endpoints: Wasp mortality after 48 h (for determination of the LR_{50}); assessment of the reproductive capacity of individually-confined females (*i.e.* numbers of parasitized aphids developing following a 24 h oviposition period).

Reference item: BAS 152 11 I (a.s.: dimethoate, 400 g/L nominal; 400.9 g/L measured).

Test rates: Control (purified water), 187.5, 375, 750, 1500 and 3000 mL

BAS 750 01 F/ha. The highest test rate was prepared by diluting 1.5 mL test substance to 200 mL with purified water. The remaining test rates were prepared diluting appropriate quantities of a stock solution (10 L/ha) to 100 mL with purified water. The reference item was applied at an application rate of 10 mL BAS 152 11 I/ha. All treatments were applied in 400 L/ha water. The treatments were sprayed on barley seedlings using a calibrated laboratory track-sprayer and left to air dry afterwards. Calibration took place in advance of applications using purified water. Three pre-weighed glass plates were sprayed and then reweighed so that the rate of deposition could be determined. Once three consecutive applications had delivered the correct deposition rate (4 mg deposit/cm^2 ± 10 %), treatments were applied. Actual deposition rates ranged between 98 and 105.8 % of nominal.

Test conditions: Exposure of adults: Temperature: 20°C - 21 C; relative humidity: 69% - 75%; photoperiod: 16 h light: 8 h dark; light intensity: 1719 lux.
Reproduction assessment: Temperature: 20°C - 22°C; photoperiod: 16 h light: 8 h dark, light intensity: 5544 lux. Food: 10% (w/v) fructose solution, sprayed onto test plants before application.

Statistics: Percentage mortality over 48 h was calculated (total number of moribund and dead insects), corrected for control mortality using Abbott's formula (1925). Fisher's Exact Test ($\alpha = 0.05$) performed on mortality data. Repellent effects of the product were measured by noting the percentage of wasps settled on the plants at five separate assessment occasions during the initial 3 h of the bioassay. Mean value for each replicate was square root-transformed prior to a normality check (Shapiro-Wilk test, $\alpha = 0.05$). One-way ANOVA and Dunnett's t-test ($\alpha = 0.05$) or Mann-Whitney U-test ($\alpha = 0.05$) on the square root-transformed reproduction data as well as the repellent effect data. Statistical analysis was carried out using SPSS 2013.

II. RESULTS AND DISCUSSION

Corrected mortalities of 0.0%, -3.4%, 3.5%, 10.3% and 13.9% were observed after 48 h in the 187.5, 375, 750, 1500 and 3000 mL BAS 750 01 F/ha treatment rates, respectively. None of these results differed significantly from the control (Fisher's Exact Test, $\alpha = 0.05$). The LR_{50} was therefore > 3000 mL BAS 750 01 F/ha in 400 L water/ha.

There were no statistically significant negative effects on reproduction and wasp-settling (repellent effects), compared to the control, at rates up to and including 3000 mL BAS 750 01 F/ha, the highest treatment rate tested (one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$) or Mann-Whitney U-test ($\alpha = 0.05$)). The results are summarised in Table B.9.5.2/4-1.

Table B.9.5.2/4-1: Effects on parasitoids (*Aphidius rhopalosiphi*) exposed to BAS 750 01 F in an extended laboratory trial

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [4] [mummies/ female]	Effects on reproduction [5] [%]
Control	--	3.3	--	24.5	--

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [4] [mummies/ female]	Effects on reproduction [5] [%]
BAS 750 01 F	187.5	3.3	0.0	~	~
	375	0.0	-3.4	~	~
	750	6.7	3.5	26.5	-8.3
	1500	13.3	10.3	27.5	-12.4
	3000	16.7	13.9	26.4	-7.9
Endpoint [mL BAS 750 01 F/ha]					
LR_{50}	> 3000				
ER_{50}	> 3000				

1) Application rate in 400 L water/ha.
2) Mortality in the individual test substance treatments after 48 hours of exposure to BAS 750 01 F on barley seedlings (Fisher's Exact Test, $\alpha = 0.05$).
3) Corrected mortality according to Abbott (1925).
4) Mean number of parasitized aphids/surviving female. Reproduction assessment was only conducted for the 3 highest application rates of the test substance where the corrected mortality was < 60% (one-way ANOVA and Dunnett's t-test, $\alpha = 0.05$, or Mann-Whitney U-test, $\alpha = 0.05$)
5) A negative value indicates a increase in reproduction relative to the control.
~ Treatment not evaluated.

The reference item caused a corrected mortality of 89.7% of the exposed organisms after 48 h.

Validity Criteria
The study meets the validity criteria as specified in Mead-Briggs *et al.* (2009).

- Control mortality did not exceed 10 % (being 3.3 %)
- Corrected mortality in the reference item treatment (10 mL/ha), within the range specified by guidance (5-20 mL/ha), was greater than 50 % (being 89.7 %)
- Control reproduction mean value was not less than 5 per female (being 24.5) and no more than two control wasps produced zero values (being zero wasps).

III. CONCLUSION

In an extended laboratory study with *Aphidius rhopalosiphi*, the LR_{50} value was determined to be > 3.0 L BAS 750 01 F/ha. The test substance caused no unacceptable effects on survival and reproduction when applied up to and including a rate of 3000 mL BAS 750 01 F/ha .

RMS Comments
The study was carried out according to GLP and follows the guideline laid out in Mead-Briggs et al. (2009) with no significant deviations. It was noted that only wasps from the treatment groups of the three highest test rates were selected for reproductive assessments. For completeness it would have been appropriate to select wasps from all treated groups, as mortality was less than 50 % in all cases. However, as there were no significant effects on reproduction in the treatment groups of the three highest test rates, the RMS evaluator does not consider it likely that significant effects would be observed in the two lower test rates. This is therefore not considered to be a significant deviation from guidance.

The agreed endpoints considered suitable for use in the risk assessment are:

- **LR_{50} (48 h) ≥ 3000 mL BAS 750 01 F /ha**
- **ER_{50} reproduction ≥ 3000 mL BAS 750 01 F /ha**

Report:	B.9.5.2/5 Vaughan R., 2015a A rate-response extended laboratory test to evaluate the effects of fresh residues of BAS 750 01 F on the green lacewing *Chrysoperla carnea* (Neuroptera, Chrysopidae) 2015/1020206
Guidelines:	Vogt H. *et al*. (2000)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: *Chrysoperla carnea* STEPH. (green lacewing), larvae (2 - 3 days old; all emerged over a 24 hour period), source: in-house culture of the test facility.

Test design: Exposure of larvae via fresh dry residues on treated bean leaves. Per replicate, the treated bean leaf was sandwiched between two clear plates, the uppermost of which possessed a 5 cm (diameter) hole through which an acrylic cylinder was fitted (internal diameter 44 mm). A fine nylon mesh placed atop each cylinder completed each test arena. The leaves were allowed to dry for approximately 30 mins after treatment before the test units were assembled and the larvae introduced.

6 treatment groups (5 test substance rates, 1 water treated control plus reference item), with 50 replicates (*i.e.* one larva per replicate) per treatment.

After introduction of the larvae, their condition was assessed every 1-3 days until they pupated. They were categorised as either *alive, abnormal pupa, dead* or *pupated.* Pupae of each treatment were collected no earlier than five days after their formation and kept in separate boxes. The number of successfully emerged adults was also recorded every 1-3 days. Adults (all emerged within 7 days) from the highest 3 test rates and the control were assessed for fecundity in polystyrene oviposition boxes. The number of eggs, laid in each box, and their viability, was recorded for two 24 hour periods within one week.

Endpoints: Pre-imaginal mortality, including determination of a LR_{50}. Reproduction assessment: number of produced eggs per female per day and hatching rate.

Reference item: BAS 152 11 I (dimethoate, 400.0 g/L nominal).

Test rates: Control (purified water); test substance: 187.5, 375, 750, 1500 and 3000 mL BAS 750 01 F/ha. A stock solution was prepared (6000 mL/ha) by diluting 6 mL test substance to 200 mL with purified water. The highest test rate was prepared by diluting 3 mL test substance to 200 mL with purified water. The lower test rates were prepared by diluting appropriate quantities of stock solution to 200 mL with purified water. The reference item was applied at a rate of 80 mL/ha. All rates were applied in 200 L water/ha. The

substances were sprayed on excised bean leaves (*P. vulgaris* var. The Prince) via calibrated laboratory track-sprayer and air dried afterwards. The sprayer was calibrated in advance of applications using purified water. Three pre-weighed plates were sprayed, then re-weighed and the rate of deposition determined. Once three consecutive applications had delivered 200 L/ha (2 mg deposit/cm^2) ± 10 %, the treatments were applied. The actual deposits during calibration ranged between 99.5 and 106.5 % of nominal.

Test conditions: Temperature: 23.6°C – 25.4°C; relative humidity: 62% - 77%; photoperiod: 16 h light: 8 h dark; light intensity: 2600 - 4000 lux. Food: larvae: *Sitotroga cerealella* eggs (UV sterilized; provided every 1-3 days), adults: artificial diet (provided 2-3 times per week; prepared as per guidance – Vogt *et al.* 2000).

Statistics: Pre-imaginal mortality was compared to that in the control using Fisher's Exact Test ($\alpha = 0.05$). The statistical software used was SPSS (2013).

II. RESULTS AND DISCUSSION

In the control a pre-imaginal mortality of 14.0% was observed. In the test substance treatments the mortality ranged between 12.0% and 24.0%. No treatment rate exhibited statistically significant effects on mortality (Fisher's Exact Test, $\alpha = 0.05$). No effects on reproduction of *Chrysoperla carnea* occurred at 750, 1500 and 3000 mL BAS 750 01 F/ha (no statistical analysis reported).

The results are summarised in Table B.9.5.2/5-1.

Table B.9.5.2/5-1: Effects on *Chrysoperla carnea* exposed to fresh dry residues of BAS 750 01 F under extended laboratory conditions

Treatment	Rate [mL/ha] [1)]	Mortality [%] [2)]	Corrected mortality [%] [3)]	Reproduction [eggs/female/day]	Hatching rate [%]
Control	--	14.0	--	36.7	94.7
BAS 750 01 H	187.5	18.0	4.7	--	--
	375	24.0	11.6	--	--
	750	14.0	0.0	27.3	93.5
	1500	20.0	7.0	32.7	95.3
	3000	12.0	-2.3	36.2	94.7
Endpoint [mL BAS 750 01 F/ha]					
LR_{50}	> 3000				

1) Application rate in 200 L water/ha.
2) Pre-imaginal mortality.
3) Corrected mortality according to Abbott (1925).

The reference item caused 100.0% mortality (100.0% corrected mortality) of exposed lacewings.

Validity Criteria
The study meets the validity criteria specified in Vogt *et al.* (2000):

- Pre-imaginal control mortality did not exceed 20 % (being 14 %)

- Control fecundity was not less than 15 eggs/female/day (being 36.7)
- Control hatching rate was not less than 70 % (being 94.7 %)
- The level of mortality in the reference item treatment was not less than 50 % (being 100 %).

III. CONCLUSION

In an extended laboratory study with BAS 750 01 F the LR_{50} for *Chrysoperla carnea* was determined to be > 3000 mL BAS 750 01 F/ha. No adverse effects on reproduction of *Chrysoperla carnea* were observed at treatment rates up to and including 3000 mL BAS 750 01 F/ha.

RMS Comments
The study was carried out according to GLP and follows the guideline specified by Vogt et al. (2000) with a couple of deviations.
The study followed a modified version of the *C. carnea* first tier laboratory study and in doing so applied a higher rate of the reference item (80 mL/ha, highest recommended in Vogt *et al.*, 2000, is 45 mL/ha). This introduces uncertainty, both into the role of the reference item and into the sensitivity of the batch of *C.carnea* used. However, regarding the effects of the test substance, it clearly shows that BAS 750 01 F is of low toxicity.
It was noted that emergent adults of the two lowest test rates (375 and 187.5 mL/ha) were not assessed for fecundity. Guidance states that if ≥ 50 % of the larvae exposed to the test substance survive and complete their metamorphosis successfully, the reproductive performance should be assessed. As mortality rates in these treatment groups were both less than 50 %, the adults should have been assessed for fecundity. However, as the adults of the three highest test rates were tested for reproductive performance and < 50 % effects were observed compared to the control for any group, it is reasonable to assume that similar results would be observed in the adults tested with lower rates of the test substance. This is therefore not considered to be a significant deviation.
Overall, the study is considered to be reliable by the RMS.

The agreed endpoints considered suitable for use in the risk assessment are:

- **LR_{50} ≥3000 mL BAS 750 01 F /ha**
- **ER_{50} reproduction ≥3000 mL BAS 750 01 F /ha**

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

Bees

Table B.9.6-1 presents the results of bee toxicity studies. For a summary of the studies with BAS 750 F, please refer to CA 8.3. Further details regarding the studies with the formulation BAS 750 01 F are provided in B.9.3.1.1.1 and B.9.3.1.1.2

Table B.9.6-1: Summary of endpoints of BAS 750 F and BAS 750 01 F to honeybees and bumblebees

Substance	Endpoint	Value	Reference
Studies on adult honeybees			
BAS 750 F	48 h acute oral LD_{50}	> 100 µg a.s./bee	B.9.3.1/1 Franke M., 2015a
	48 h acute contact LD_{50}	> 100.0 µg a.s./bee	
	10 d chronic LD_{50}	> 110.5 µg a.s./bee/day	B.9.3.1/5 Kleebaum K., 2015a
	10 d chronic LC_{50}	> 2.562 g a.s./kg food	
	10 d chronic NOED	≥ 110.5 µg a.s./bee/day	
	10 d chronic NOEC	≥ 2.562 g a.s./kg food	
BAS 750 01 F	48 h oral LD_{50}	409.6 µg **BAS 750 01 F** /bee	B.9.5.1/2 Franke M., 2015a
	96 h contact LD_{50}	296.4 µg **BAS 750 01 F** /bee	
Studies on honeybee larvae			
BAS 750 F	8 d LD_{50}	43.9 µg a.s./larva	B.9.3.1/6 Kleebaum K., 2015b
	8 d NOED	29.7 µg a.s./larva	
	8 d LC_{50}	1.295 g a.s./kg food	
	8 d NOEC	0.875 g a.s./kg food	
Studies on adult bumblebees			
BAS 750 F	96 h oral LD_{50}	> 195.4 µg a.s./bee	B.9.3.1/2 Amsel K., 2015a**
	96 h contact LD_{50}	> 200.0 µg a.s./bee	

****Studies provided as additional information but not used in risk assessment.**

Exposure

Table B.9.6-2: Proposed use pattern of BAS 750 01 F

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				BAS 750 F [g a.s./ha]	BAS 750 01 F [L/ha]
cereals	30 - 69	2	14	150	1.5

Applications of pesticides can potentially result in exposure of bees either through direct over-spray, or by contact with residues on plants while bees are foraging for food. However, cereals are a crop of low attractiveness for foraging bees. Hence, the following risk assessment can be regarded as worst-case scenario. The maximum single application rate for the active substance BAS 750 F and representative formulation BAS 750 01 F is used for the risk assessment.

Risk assessment for bees

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

Standing Committee on Plants, Animals, Food and Feed.

The acute risk to honeybees from the use of BAS 750 01 F was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients [*EPPO/OEPP, 2003: Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145*] as follows:

$$\text{Hazard Quotient (HQ)} = \frac{\text{Maximum application rate [g formulation/ha]}}{\text{Acute LD}_{50}\ [\mu\text{g formulation/bee}]}$$

HQs for honeybees were calculated for oral exposure and contact exposure to BAS 750 F and BAS 750 01 F. An HQ < 50 indicates low risk to honeybees in the field.

Table B.9.6-3: Risk to honeybees from exposure to BAS 750 F and BAS 750 01 F considering the worst-case application rate

Test substance	Application rate [g/ha]	Endpoint	LD_{50} [µg/bee]	Hazard quotient HQ	Trigger
Risk assessment on adult honeybees					
BAS 750 F	150	48 h oral	> 100.0	< 1.5	50
		48 h contact	> 100.0	< 1.5	
BAS 750 01 F	1489.5 *	48 h oral	409.6	3.64	
		96 h contact	296.4	5.03	

* Taking into account the density of BAS 750 01 F of 0.993 g/cm^3.

The calculated HQs for acute oral and acute contact exposure of honeybees to BAS 750 F and BAS 750 01 F are below the trigger value of 50 with a high margin of safety. Therefore, low acute risk to honeybees is expected after application of BAS 750 01 F according to good agricultural practice.

The data pertaining to chronic honeybee toxicity, toxicity to honeybee larvae and acute toxicity to bumblebees provided by the applicant has been evaluated in Volume 3 CA section B.9.3 and summarised in the table below:

Substance	Endpoint	Value	Reference
Studies on adult honeybees			
BAS 750 F	10 d chronic LD_{50}	> 110.5 µg a.s./bee/day	B.9.3.1/5 Kleebaum K., 2015a
	10 d chronic LC_{50}	> 2.562 g a.s./kg food	
	10 d chronic NOED	≥ 110.5 µg a.s./bee/day	
	10 d chronic NOEC	≥ 2.562 g a.s./kg food	
Studies on honeybee larvae			
BAS 750 F	8 d LD_{50}	43.9 µg a.s./larva	B.9.3.1/6 Kleebaum K., 2015b
	8 d NOED	29.7 µg a.s./larva	
	8 d LC_{50}	1.295 g a.s./kg food	
	8 d NOEC	0.875 g a.s./kg food	
Studies on adult bumblebees			
BAS 750 F	96 h oral LD_{50}	> 195.4 µg a.s./bee	B.9.3.1/2 Amsel K., 2015a**
	96 h contact LD_{50}	> 200.0 µg a.s./bee	

Under Commission Regulation No 283/2013 and 284/2013, chronic toxicity to adult honeybees and toxicity to honeybee brood need to be addressed. However there is currently no agreed risk assessment scheme available to assess the risk for bees based on chronic adult and/or larvae data.
Therefore this data has not been used to assess the risk to this organism group. The risk to bees is concluded to be acceptable based on the data available for acute toxicity to honeybees, as per current noted guidance.

Overall, taking into account all available data, the use of BAS 750 01 F according to good agricultural practice presents low risk to bees and their colonies.

Other Non-Target Arthropods

For other non-target arthropods (NTAs), only the risk of the formulation is considered. The applicant has provided study data for NTAs which have been evaluated and commented on in the previous section. The endpoints are summarised in the table below:

Available study endpoints

Reference	**Organism**	**Study Type**	**LR_{50} (ml/ha)**	**ER_{50} (reproduction, ml/ha)**
Fallowfield L. 2015a	*T. pyri*	Tier 1	769.1	none
Stevens J. 2015a	*A. rhopalosiphi*		95.4	none
Fallowfield L. 2015b	*T. pyri*	Extended lab	>3000	>3000
Stevens J. 2015b	*A. rhopalosiphi*		>3000	>3000
Vaughan R. 2015a	*C. carnea*		>3000	>3000

Exposure

GAP table for BAS 750 01 F

Crop	**Target**	**Application**			**Application rate**		
		Method/ Kind	Timing/ Growth stage of crop & season	Max. number of (min. interval between) applications a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max
Cereals	*Septoria tritici*	Foliar spray	BBCH 30-69	a) 2 (14) b) 2 (14)	a) 1.5 L/ha b) 3.0 L/ha	150 g as/ha 300 g as/ha	100 - 300

The guidance document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-Target Arthropods from the ESCORT 2 workshop (Candolphi *et al.*, 2000) uses the following equations to calculate hazard quotients for in-field and off-field exposure scenarios:

In field exposure = App. Rate x MAF
Where:
App. Rate = 1500 mL/ha
MAF (2 applications, default value for foliar application) = 1.7*

In field exposure = (1500 x 1.7) = **2550 mL/ha**

Off field exposure = (App. Rate x MAF x Drift x Correction Factor)
App. Rate = 1500 mL/ha
MAF (2 apps, default value for foliar app) = 1.7
Drift value (2 applications, field crops) = 2.38/100 = 0.0238*
Correction Factor (first tier studies – takes risk to more sensitive off-target species into account) = 10

Off field exposure = (1500 x 1.7 x 0.0238 x 10) = **606.9 mL/ha**

*Within the guidance document the MAF is obtained from Appendix 3 and the drift factor is obtained from Appendix 4.

Risk Assessment
First tier risk assessment depends on LR50 values (ESCORT 2 Guidance) and uses the Hazard Quotient (HQ) approach. Based on the above equations and the input parameters the HQ values for the indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* are given in tables 1 and 2 for in-field and off-field scenarios, respectively.

Table 1: In-field HQ = In field exposure/LR50

Organism	**Study Type**	**LR_{50} (ml/ha)**	**In field exposure (mL/ha)**	**HQ**	**Trigger**
T. pyri	Tier 1	769.1	2550	**3.32**	2
A. rhopalosiphi		95.4		**26.73**	2

Both HQ values are greater than the trigger value of 2. Therefore the in-field risk requires further consideration.

Table 2: Off-field HQ = Off field exposure/(LR_{50} x Vegetation Distribution Factor*)

Organism	Study Type	LR_{50} (ml/ha)	Off field exposure (mL/ha)	VDF*	HQ	Trigger
T. pyri	Tier 1	769.1	606.9	10	0.079	2
A. rhopalosiphi		95.4			0.64	2

*Default VDF of 10 is applied when using endpoints from 2D studies

Both HQ values are lower than the trigger value of 2. Therefore the off-field risk requires no further consideration.

In-field risk refinement

Where the in-field HQ for at least 1 species tested is greater than 2, but the off-field HQ for both species tested are less than 2, further testing on the species that failed and 1 additional crop-relevant species are required. These extended laboratory tests involve more realistic exposure conditions, typically by exchanging the 'glass plate' exposure apparatus with a leaf disc substrate (2D study) or by spraying a whole plant over which the organisms are contained (3D study). The applicant has provided data for both species that failed the in-field risk assessment at tier 1 (*T. pyri* and *A. rhopalosiphi*) and 1 additional crop-relevant species (*C. carnea*). These data have been evaluated by the RMS and found to be suitable for the risk assessment. Therefore the applicant has met the data requirements for refining the in-field risk to NTAs.

With extended laboratory study data, the HQ approach is not considered appropriate and instead the endpoints (LR_{50} and ER_{50}) derived are compared with the predicted in-field exposure. If the endpoints are greater than the predicted in-field exposure, then an acceptable risk can be concluded.

Organism	Study Type	LR_{50} (ml/ha)	ER_{50} (ml/ha) - Fecundity	In-field exposure (mL/ha)	Toxicity > Exposure?
T. pyri	Extended lab	>3000	>3000	2550	Yes
A. rhopalosiphi		>3000	>3000		Yes
C. carnea		>3000	>3000		Yes

The predicted in-field exposure is lower than the LR_{50} and ER_{50} derived from extended laboratory studies. Therefore, the in-field risk to non-target arthropods can be considered resolved.

Overall conclusion:

It is concluded that low risk for non-target arthropods is expected from the use of BAS 750 01 F according to the proposed use pattern. According to the current guidance, effects on non-target arthropods are considered acceptable in in-field and off-field habitats.

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1. Earthworms

Report: B.9.7.1/1
Ganβmann M., 2015a
Effects of BAS 750 01 F on reproduction and growth of earthworms *Eisenia fetida* in artificial soil with 10% peat
2015/1000884
Guidelines: OECD 222 (2004), ISO 11268-2 (2012)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: *Eisenia fetida* (earthworm); adult worms with clitellum, and weight of 300 - 540 mg, approximately 9 months old (age difference ≤ 4 weeks), source: in-house culture. The worms were acclimatised for 1 day in test conditions.

Test design: 56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test substance were mixed homogeneously into the soil; 6 treatment groups were set up (5 concentrations of the test substance and untreated control) with 4 replicates for the test substance treatment groups and 8 replicates for the control and 10 worms per replicate; assessment of worm mortality, behavioural 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, weight change, feeding activity and reproduction rate.

Reference item: Carbendazim (499 g/kg). The effects of the reference item were investigated in a separate study (September 2014). Significant effects on reproduction were observed at concentrations of 1.95 and 2.94 mg a.s./kg dsw, which falls within the range described by guidance (OECD 222; 1-5 mg a.s./kg dsw). This therefore confirms the sensitivity of the test system.

Test concentrations: Control, 32.8, 41.0, 51.2, 64.0 and 80.0 mg BAS 750 01 F/kg dry soil. A stock solution was prepared by weighing 570 mg of the product and diluting to 1200 g with deionised water (final concentration 0.475 mg f.p/g). Appropriate quantities of the stock solution were added to 2050 g soil (dry weight) and mixed in a laboratory mixer to prepare the range of test concentrations before being split into replicates (see below).

Test conditions: Artificial soil according to OECD 222 (10 % peat, 20 % Kaolin clay, 69.5 % quartz sand, 0.5 % $CaCO_3$). The soil was moistened to approximately half the final water content 1 day prior to application of the test substance, and allocated to plastic boxes in 500 g (dry weight) portions (1 per replicate), so

that each test unit had a soil depth of approximately 4-5 cm.
pH 5.9 – 6.2 at test initiation, pH 6.2 – 6.4 at test termination; water content: 52.9% – 55.9% of max. water holding capacity (WHC) at test initiation, 55.2% – 59.0% of WHC at test termination; temperature: 18 °C – 22 °C; photoperiod: 16 hours light: 8 hours dark, light intensity: 400 – 800 lux. Food was finely ground cattle manure, applied weekly during the first 28 days of the test, at a rate of 5 g/test unit and moistened with 2-3 g deionised water.

Statistics: Body weight change and reproduction data were tested for normal distribution and homogeneity of variance ($\alpha = 0.05$) using the Shapiro-Wilk's test and the Levene's test, respectively. Williams t-test ($\alpha = 0.05$, two-sided for weight changes and one-sided smaller for reproduction). The software used to perform the statistical analysis was ToxRat Professional (Version 2.10.05, ® ToxRat Solutions GmbH).

II. RESULTS AND DISCUSSION

BAS 750 01 F did not show any statistically significant effects on survival, body weight and reproduction. No mortality was observed at any test substance concentration or in the control. The body weight changes were not statistically significantly different compared to the control in any test substance treated group (Williams t-test, $\alpha = 0.05$, two-sided).

The reproduction rates were not statistically significantly different compared to those in the control up to and including the highest concentration of 80 mg BAS 750 01 F/kg dry soil (Williams-t test, $\alpha = 0.05$, one-sided,). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. As this test showed no effects up to and including the highest test concentration (highest reproduction has been observed at the highest test concentration of 80.0 mg/kg dry soil), it is not feasible to derive a proper EC_{10} and hence no such values are presented. The results are summarised in Table B.9.7.1/1-1.

Table B.9.7.1/1-1: Effects of BAS 750 01 F on *Eisenia fetida* in a 56-day reproduction study

BAS 750 01 F [mg/kg dry soil]	**Control**	**32.8**	**41.0**	**51.2**	**64.0**	**80.0**
Mortality (28 d) [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change (28 d; increase) [%]	61.8	55.6	64.3	53.4	52.1	53.0
Number of juveniles (56 d)	286	282	269	243	262	301
Coefficient of variation (Reproduction; %)	8.7	9.9	14.8	23	16	6.6
Reproduction [% of control] (56 d)	--	98.5	93.9	84.7	91.6	105.1
Endpoints [mg BAS 750 01 F/kg dry soil]						
NOEC (28 d, $_{\text{mortality}}$)	≥ 80					
NOEC (28 d, $_{\text{weight change}}$)	≥ 80					
NOEC (56 d, $_{\text{reproduction}}$)	≥ 80					

Validity Criteria
The study meets the validity criteria specified in OECD 222:

- Control number of juveniles per replicate was greater than 30 (being at least 251)
- Control coefficient of variation for reproduction was less than not more than 30 % (being 8.7 %)
- Control adult mortality over the initial 4 weeks was no more than 10 % (being 0 %)

III. CONCLUSION

In a 56-day reproduction study with BAS 750 01 F on earthworms (*Eisenia fetida*), the NOEC for mortality, body weight, reproduction and feeding activity was determined to be ≥ 80 mg BAS 750 01 F/kg dry soil, the highest concentration tested.

RMS Comments
The study was carried out according to the principles of GLP and follows the guidelines OECD 222 and ISO 11268-2 with no deviations noted. It was noted that although the study reports less than 5 % deviation from nominal test concentrations, the analytical method and results are not presented within the study report. Actual test concentrations in the soil are not specified as required in OECD guidance therefore the RMS does not consider this to be an important omission of data.

The agreed endpoint suitable for use in the risk assessment is:
56 day NOEC ≥ 80 mg BAS 750 01 F/kg dsw (equivalent to 7.9 mg a.s./kg dsw, based on the density of the formulation being 0.993 g/cm^3 and the concentration of the active substance being 98.9 g/L)

Report:	B.9.7.1/2 Hamberger A., 2015a BAS 752 AM F - A field study to investigate effects on earthworm fauna in Southern Germany 2015/1000261
Guidelines:	ISO 11268-3 (1999), DIN ISO 23611-1 (2006), Kula *et al.* (2006) - Technical Recommendations for the Update of the ISO Earthworm Field Test Guideline (ISO 11268-3)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance:	BAS 752 AM F, batch no. FD-140116-0001; contents of a.s.: BAS 750 F: 100.0 g/L nominal (97.8 g/L analysed); fluxapyroxad (BAS 700 F): 50.0 g/L nominal (48.8 g/L analysed) density: 0.996 g/cm^3.
Reference item:	Twist WP® (a.s.: carbendazim, 600 g/kg nominal, 583.0 g/kg analysed).

B. STUDY DESIGN

Test species:	Naturally occurring field population of earthworms comprising all mobile stages (juvenile and adult) including *Aporrectodea caliginosa* (34.5 worms/m^2 pre-treatment), *Aporrectodea limicola, Aporrectodea rosea* (10.8 worms/m^2 pre-treatment), *Dendrodrilus rubidus. Lumbricus castaneus, Lumbricus rubellus. Lumbricus terrestris* (16.6 worms/m^2 pre-treatment),

	Octolasion cyaneum, *Octolasion lacteum*, juvenile tanylobous (101.9 worms/m^2 pre-treatment), juvenile epilobous (257 worms/m^2 pre-treatment), tanylobous front ends and epilobous front ends
Test site:	Arable field site near Vaihingen-Enz, Southern Germany. The selected area did not receive an application of any crop protection product during the study apart from the test substance and the reference item, including the control plots. No fertilisers or pesticides had been applied to the test site in the four years preceding the start of the study. The test soil had the following characteristics: silt loam soil; C_{org} = 1.4 %; pH = 5.23; Water Holding Capacity (WHC): 32 g/100g dry soil weight (dsw).
Test design:	Randomised block design, the study area was divided into 20 plots of 144 m^2 (12 m x 12 m) arranged in 23 rows. Each plot was surrounded by a guard row of at least 5 m. Assignment of the treatment groups was based on the pre-sampling data, such that initial earthworm populations at the trial start were equal in the 4 blocks; 5 treatment groups (3 test substance treatment groups, control and a reference item) each with 4 replicates. 8 samples were taken per sampling date per replicate (32 samples per treatment per sampling occasion).
Endpoints:	Total abundance and biomass of earthworms.
Test rates:	Single application of each treatment to bare soil. After application a grass clover mixture was sown manually and mown from time to time (cuttings were left on the plots). The field site was irrigated 3 days prior to application and directly afterwards. Additionally irrigation took place 2, 4, 6, 7, 9 and 15 days after application (total 51.6 mm; measured with a calibrated rain gauge), which in combination with natural rainfall ensured sufficient soil moisture levels such that earthworms were active and exposed to the treatments. Untreated control (tap water); Treatment group 1: BAS 752 AM F applied at 2.0 L/ha (195.6 g BAS 750 F/ha nominal); Treatment group 2: BAS 752 AM F applied at 4.0 L/ha (391.2 g BAS 750 F/ha nominal); Treatment group 3: BAS 752 AM F applied at 8.0 L/ha (783.4 g BAS 750 F/ha nominal); Treatment group 4: reference item applied at 17.2 kg/ha (10 kg carbendazim/ha). All treatment groups were applied in 300 L water/ha. Application was performed using a calibrated boom sprayer with a spray width of 6 m and with 12/DG11002 VS Teejeet nozzles. The spraying equipment was calibrated by measuring nozzle outputs for a given time over three runs, with a deviation between outputs and different runs of < 5 %. Actual applied rates per plot of the test substance and reference item were within -3.5 – 8 % of the target application rate (300 L/ha). Soil core sampling for analytical verification was performed on the day of application (after application) in the test substance and control plots. 10 soil cores (50 mm diameter x 100 mm depth) were taken per plot. Per plot, the soil from these cores was mixed and divided into two samples for residue analysis of the active substances BAS 700 F (Limit of Quantification, LOQ

= 0.001 mg/kg) and BAS 750 F (LOQ = 0.002 mg/kg). BAS 700 F residues after application were analysed by Petri dish analysis (LOQ = 0.001 mg/kg).

Sampling method: Surface monitoring for mortality: daily from day 1 until day 3 after application. The soil surface was checked for dead earthworms by visual observation of two monitoring areas per plot in all replicates of the control and test substance plots. The monitoring areas of 1 m^2 (total 2 m^2 per plot) were placed in pre-assigned central locations within each plot. Assessments were conducted within the same areas.

Population sampling: Defined areas were sampled to assess earthworm populations 14 - 10 days before and three times after the application (approx. 1, 6 and 12 months after application). Earthworms were sampled from four 0.5 m² sampling areas per plot per sampling occasion with a sampling depth of 20 cm. The sampling method was hand sorting combined with formalin extraction (0.2 % formaldehyde, 20 L/m^2) in the excavated hole. Earthworms were collected for 30 mins after formalin treatment. They were then preserved in watertight containers containing 80 % alcohol. The worms were dried on filter paper prior to weighing.

Sampling dates: Pre-sampling: 24.03.2014 – 28.03.2014 (14 – 10 Days Before Application, DBA);
1st sampling: 12.05.2014 – 16.05.2014 (35 – 39 Days after application, DAA);
2nd sampling: 06.10.2014 – 14.10.2014 (182 – 190 DAA);
3rd sampling: 23.04.2015 – 05.05.2015 (381 – 393 DAA).

Test conditions: Climatic parameters measured on day of application, each monitoring and each sampling day. Mean air temperatures during application: 20.0 °C – 21.7 °C, Mean relative air humidity during application: 35.5% - 42.3% (soil dry at time of application). Climatic conditions during the study period are summarised in the table below:

Month	Mean air temperature	Max. air temperature	Min. air temperature	Precipitation	Mean air humidity	Soil humidity 5 cm
	°C	°C	°C	mm	volume %	volume %
March 2014	8.2*	22.8*	-2.3*	9.0*	69.3*	47.3*
April 2014	12.0*	24.9*	-1.0*	41.2*	70.7*	35.7
May 2014	13.3[a)]	28.3 [a)]	2.3 [a)]	83.6 [a)]	77.4 [a)]	37.6
June 2014	17.8	34.4	6.5	20.2	71.2	24.6
July 2014	19.1	33.6	9.0	131.2	78.2	31.1
August 2014	16.3	28.4	7.0	111.0	84.0	31.2
September 2014	15.1	25.7	4.7	83.8	90.7	38.6
October 2014	12.2	24.5	2.2	62.2	93.8	39.1
November 2014	6.6	17.8	0.3	63.2	96.7	40.5
December 2014	3.2[b)]	11.2 [b)]	-6.6 [b)]	21.2 [b)]	95.7 [b)]	41.3
Januar 2015	2.3	14.3	-4.8	49.0	94.4	43.2
February 2015	0.1	13.1	-7.9	24.6	90.0	43.2
March 2015	6.1	17.7	-2.6	50.8	79.8	40.8
April 2015	9.9	24.3	-2.3	51.6	70.2	38.7
May 2015	13.6	28.1	3.1	65.4	80.3	35.0

*Data taken from own weather station in Niefern

[a)]Missing data taken from own weather station in Niefern (period: 01 May – 08 May 2014, 10 – 11 May 2014 and for air humidity: 12 – 14 May 2014)

[b)]Missing data taken from own weather station Niefern (period: 29 – 31 Dec 2014)

Statistics: Descriptive statistics. ANOVA followed by Dunnett-t-test (two-sided, $\alpha = 0.05$) for homogenous data. If neither homogeneity of variance (Levene's test; $\alpha = 0.05$) nor normality (Shapiro-Wilk's test; $\alpha = 0.05$) were observed a Kruskal-Wallis test was used. The test was followed by a Wilcoxon test for pair-wise comparisons of the individual treatments and a correction of p-values according to Bonferroni-Holms procedure (Bonferroni-U-Test). Student-t-test or Satterthwaite-t-test for reference item data (two-sided, $\alpha = 0.05$). Statistical analysis carried out using SAS version 9.3.

II. RESULTS AND DISCUSSION

Surface monitoring on days 1-3 after application showed that there was no acute primary effect on earthworms by BAS 752 AM F. No dead earthworms were found on the soil surface of the test substance monitoring areas. After application of BAS 752 AM F no significant reductions in total earthworm abundance and biomass were identified in all test substance treatments at 35, 182 or 381 DAA. No statistically significant reductions compared to the control could be found for the dominant species *A. caliginosa, A. rosea* and *L. terrestris* at any of the sampling dates (ANOVA followed by Dunnett-t-test, two-sided, $\alpha = 0.05$).

The results are summarised in Table B.9.7.1/2-1. Also included are data for the three main species of concern (*A. caliginosa, A. rosea* and *L. terrestris*) and adult:juvenile ratios (Tables B.9.7.1/2-2 to 5).

Table B.9.7.1/2-1: Summary of total earthworm abundance and biomass in a field study with BAS 752 AM F

Treatment	Pre-sampling 24.03.2014 – 28.03.2014 [1)]	First sampling 12.05.2014 – 16.05.2014	Second sampling 06.10.2014 – 14.10.2014	Third sampling 23.04.2015 – 05.05.2015
Total earthworm abundance [individuals/m²] ± SD				
Control	431.6 ± 72.1	385.3 ± 44.0	682.5 ± 79.6	485.8 ± 102.9
2.0 L BAS 752 AM F/ha	439.4 ± 86.5	389.9 ± 34.9	625.3 ± 68.3	490.0 ± 77.8
% of control	**101.8**	**101.2**	**91.6**	**100.9**
4.0 L BAS 752 AM F/ha	423.8 ± 76.4	402.3 ± 71.3	624.3 ± 99.6	431.8 ± 101.9
% of control	**98.2**	**104.4**	**91.5**	**88.9**
8.0 L BAS 752 AM F/ha	424.6 ± 90.7	387.3 ± 85.1	639.4 ± 59.6	451.5 ± 45.9
% of control	**98.4**	**100.5**	**93.7**	**92.9**
Reference item: 10.0 kg Carbendazim/ha	414.6 ± 111.0	105.9 ± 50.3*	267.4 ± 59.1*	226.6 ± 74.7*
% of control	**96.1**	**27.5**	**39.2**	**46.7**
Total earthworm biomass [g/m²] ± SD				
Control	153.5 ± 42.7	161.4 ± 30.3	255.1 ± 50.7	211.4 ± 26.8
2.0 L BAS 752 AM F/ha	143.0 ± 5.7	166.3 ± 30.5	230.6 ± 36.7	191.1 ± 18.5
% of control	**93.1**	**103.0**	**90.4**	**90.4**
4.0 L BAS 752 AM F/ha	143.3 ± 9.1	157.3 ± 42.7	258.2 ± 25.4	199.2 ± 41.8
% of control	**93.4**	**97.5**	**101.2**	**94.3**
8.0 L BAS 752 AM F/ha	149.1 ± 42.5	172.4 ± 28.2	252.7 ± 39.1	213.6 ± 37.5
% of control	**97.1**	**106.8**	**99.1**	**101.1**
Reference item: 10.0 kg Carbendazim/ha	150.8 ± 35.8	34.5 ± 8.0*	102.2 ± 13.7*	128.7 ± 24.4*
% of control	**98.3**	**21.4**	**40.0**	**60.9**

[1)] Prior to application.

Table B.9.7.1/2-2 – Means ± SD of the total abundance and biomass of *Aporrectodea caliginosa* adults in the control and the test substance treatments at each sampling.

Sampling	Abundance				
	C	T1	T2	T3	R
	mean number [n/m²] ± SD (% of Control)				
Sampling 1 14 – 10 DBA	37.8 ± 9.5	29.4 ± 11.3 (77.8 %)	34.5 ± 9.5 (91.4 %)	31.5 ± 15.2 (83.4 %)	39.6 ± 7.5 (105.0 %)
Sampling 2 35 – 39 DAA	35.9 ± 10.1	34.9 ± 4.8 (97.2 %)	35.5 ± 2.1 (99.0 %)	38.6 ± 14.4 (107.7 %)	19.4 ± 7.3* (54.0 %)
Sampling 3 182 – 190 DAA	76.1 ± 15.9	79.5 ± 18.8 (104.4 %)	67.4 ± 14.2 (88.5 %)	76.5 ± 4.0 (100.5 %)	51.1 ± 9.7* (67.2 %)
Sampling 4 381 – 393 DAA	31.8 ± 22.8	45.5 ± 14.0 (143.3 %)	30.8 ± 8.7 (96.9 %)	23.5 ± 16.1 (74.0 %)	50.1 ± 14.9 (157.9 %)
Sampling	**Biomass**				
	C	**T1**	**T2**	**T3**	**R**
	mean weight [g/m²] ± SD (% of Control)				
Sampling 1 14 – 10 DBA	18.2 ± 3.3	14.2 ± 4.1 (77.7 %)	15.1 ± 3.5 (82.7 %)	15.2 ± 7.4 (83.2 %)	19.1 ± 6.4 (104.6 %)
Sampling 2 35 – 39 DAA	15.5 ± 4.8	14.9 ± 2.3 (96.1 %)	15.0 ± 1.0 (96.9 %)	17.1 ± 5.8 (110.2 %)	6.0 ± 2.1* (38.4 %)
Sampling 3 182 – 190 DAA	27.2 ± 7.0	26.1 ± 6.5 (95.9 %)	22.4 ± 5.1 (82.2 %)	25.4 ± 1.3 (93.4 %)	20.1 ± 4.5 (73.9 %)
Sampling 4 381 – 393 DAA	9.8 ± 7.5	14.7 ± 4.8 (149.2 %)	8.9 ± 3.2 (90.8 %)	7.2 ± 5.0 (73.0 %)	17.5 ± 5.6 (177.6 %)

Sampling 1 = Pre-treatment sampling (24 Mar 2014 – 28 Mar 2014, 14 - 10 DBA)
Sampling 2 = 1st post-treatment sampling (12 May 2014 – 16 May 2014, 35 - 39 DAA)
Sampling 3 = 2nd post-treatment sampling (06 Oct 2014 – 14 Oct 2014, 182 - 190 DAA)
Sampling 4 = 3rd post-treatment sampling (23 Apr 2015 – 05 May 2015, 381 - 393 DAA)

* = statistically significant difference from control ($p \leq 0.05$)

DBA = days before application
DAA = days after application

Table B.9.7.1/2-3 – Means ± SD of the total abundance and biomass of *Aporrectodea rosea* adults in the control and the test substance treatments at each sampling.

Values in brackets represent the percentages from control values.

Sampling	Abundance				
	C	T1	T2	T3	R
	mean number [n/m²] ± SD (% of Control)				
Sampling 1 14 – 10 DBA	9.0 ± 4.1	9.9 ± 2.2 (109.7 %)	9.9 ± 3.5 (109.7 %)	13.6 ± 4.8 (151.4 %)	11.6 ± 2.2 (129.2 %)
Sampling 2 35 – 39 DAA	13.6 ± 7.3	16.9 ± 5.8 (123.9 %)	16.4 ± 6.4 (120.2 %)	13.1 ± 6.4 (96.3 %)	2.4 ± 3.1* (17.4 %)
Sampling 3 182 – 190 DAA	20.3 ± 4.3	21.6 ± 3.9 (106.8 %)	17.1 ± 1.5 (84.6 %)	24.1 ± 8.1 (119.1 %)	9.4 ± 3.9* (46.3 %)
Sampling 4 381 – 393 DAA	27.9 ± 6.6	25.8 ± 9.7 (92.4 %)	20.3 ± 3.4 (72.6 %)	28.1 ± 10.9 (100.9 %)	14.8 ± 6.7* (52.9 %)
Sampling	**Biomass**				
	C	**T1**	**T2**	**T3**	**R**
	mean weight [g/m²] ± SD (% of Control)				
Sampling 1 14 – 10 DBA	1.6 ± 0.4	1.7 ± 0.7 (109.4 %)	1.3 ± 0.6 (83.1 %)	2.2 ± 0.5 (141.7 %)	1.8 ± 0.2 (115.7 %)
Sampling 2 35 – 39 DAA	1.7 ± 0.6	2.3 ± 0.9 (140.7 %)	2.0 ± 1.0 (118.7 %)	2.1 ± 1.0 (127.3 %)	0.4 ± 0.4* (22.1 %)
Sampling 3 182 – 190 DAA	2.4 ± 0.5	2.5 ± 0.2 (108.1 %)	1.9 ± 0.2 (81.2 %)	2.7 ± 0.7 (113.3 %)	1.2 ± 0.5* (52.7 %)
Sampling 4 381 – 393 DAA	3.1 ± 0.8	3.1 ± 1.5 (100.5 %)	2.3 ± 0.3 (75.3 %)	3.0 ± 1.1 (96.6 %)	2.0 ± 0.8 (65.1 %)

Sampling 1 = Pre-treatment sampling (24 Mar 2014 – 28 Mar 2014, 14 - 10 DBA)
Sampling 2 = 1st post-treatment sampling (12 May 2014 – 16 May 2014, 35 - 39 DAA)
Sampling 3 = 2nd post-treatment sampling (06 Oct 2014 – 14 Oct 2014, 182 - 190 DAA)
Sampling 4 = 3rd post-treatment sampling (23 Apr 2015 – 05 May 2015, 381 - 393 DAA)

* = statistically significant difference from control ($p \leq 0.05$)

DBA = days before application
DAA = days after application

Table B.9.7.1/2-4 – Means ± SD of the total abundance and biomass of *Lumbricus terrestris* adults in the control and the test substance treatments at each sampling.

Sampling	Abundance				
	C	T1	T2	T3	R
	mean number [n/m²] ± SD (% of Control)				
Sampling 1 14 – 10 DBA	17.5 ± 7.4	15.6 ± 1.9 (89.3 %)	16.0 ± 3.4 (91.4 %)	16.1 ± 5.1 (92.1 %)	17.6 ± 5.9 (100.7 %)
Sampling 2 35 – 39 DAA	15.8 ± 5.4	18.6 ± 4.9 (118.3 %)	15.6 ± 1.8 (99.2 %)	18.6 ± 4.2 (118.3 %)	5.4 ± 1.0* (34.1 %)
Sampling 3 182 – 190 DAA	31.3 ± 10.8	32.5 ± 5.8 (104.0 %)	35.5 ± 5.1 (113.6 %)	35.9 ± 11.2 (114.8 %)	8.3 ± 2.4* (26.4 %)
Sampling 4 381 – 393 DAA	33.0 ± 8.2	30.0 ± 7.0 (90.9 %)	34.4 ± 9.8 (104.2 %)	35.8 ± 6.3 (108.3 %)	14.9 ± 7.2* (45.1 %)
Sampling	**Biomass**				
	C	**T1**	**T2**	**T3**	**R**
	mean weight [g/m²] ± SD (% of Control)				
Sampling 1 14 – 10 DBA	60.8 ± 27.3	54.4 ± 9.7 (89.5 %)	56.1 ± 9.9 (92.2 %)	60.4 ± 26.0 (99.3 %)	60.4 ± 21.2 (99.3 %)
Sampling 2 35 – 39 DAA	54.3 ± 20.9	64.3 ± 17.2 (118.4 %)	55.7 ± 11.6 (102.4 %)	66.3 ± 10.9 (122.0 %)	12.5 ± 3.8* (23.0 %)
Sampling 3 182 – 190 DAA	106.9 ± 40.5	103.6 ± 14.5 (96.9 %)	124.7 ± 20.6 (116.7 %)	112.9 ± 38.3 (105.6 %)	31.7 ± 8.9* (29.7 %)
Sampling 4 381 – 393 DAA	105.3 ± 31.7	88.6 ± 15.3 (84.2 %)	108.8 ± 33.6 (103.3 %)	108.1 ± 24.0 (102.7 %)	52.8 ± 27.1* (50.1 %)

Sampling 1 = Pre-treatment sampling (24 Mar 2014 – 28 Mar 2014, 14 - 10 DBA)
Sampling 2 = 1st post-treatment sampling (12 May 2014 – 16 May 2014, 35 - 39 DAA)
Sampling 3 = 2nd post-treatment sampling (06 Oct 2014 – 14 Oct 2014, 182 - 190 DAA)
Sampling 4 = 3rd post-treatment sampling (23 Apr 2015 – 05 May 2015, 381 - 393 DAA)

* = statistically significant difference from control ($p \leq 0.05$)

DBA = days before application
DAA = days after application

Table B.9.7.1/2-5 – Adult: juvenile ratio and % adult earthworms in control and treatment plots

Sampling	Control		T1 BAS 752 AM F at a rate of 2.0 L product ha^{-1}		T2 BAS 752 AM F at a rate of 4.0 L product ha^{-1}		T3 BAS 752 AM F at a rate of 8.0 L product ha^{-1}	
	adult: juvenile ratio	% adults	adult: juvenile ratio	% adults	adult: juvenile ratio	% adults	adult: juvenile ratio	% adults
1	0.19	15.8	0.15	13.3	0.18	15.6	0.18	15.4
2	0.24	19.7	0.28	22.1	0.24	19.2	0.27	21.0
3	0.27	21.5	0.32	24.3	0.31	23.6	0.32	24.5
4	0.29	22.5	0.34	25.3	0.36	26.7	0.28	22.0

Please note that no consideration of the adult: juvenile ratio was made for specific species, nor were the effects of the reference item on this parameter reported.

Validity Criteria:

- The reference item reduced total earthworm abundance by 72.5% at 35 DAA, 60.8% at 182 DAA and 53.3% at 381 DAA. This meets recommendations of guidance (Kula *et al.* 2006 for application rates of 6.0-10.0 kg/ha (at least 40 % reduction in abundance and/or biomass compared to control at least at one sampling date).
- Sufficient abundance of earthworms prior to test start was confirmed, being greater than 60 individuals per m^2 in all control plots (426.8 individuals/m^2). The proportion of ecologically important species such as *Lumbricus terrestris* (25.1 %) and *Aporrectodea caliginosa* (52.3 %) were more than 10 % of total adult earthworm abundance.
- Analytical verification of application rates revealed that mean residue levels in soil cores (BAS 700 F = 69.1 – 86.5 % of nominal; BAS 750 F = 85.1 – 90.1 % of nominal) were within the recommended range (50 – 150 % of nominal). The method of analysis was confirmed as validated (III CP B.5.1.2). The results are summarised in the table below:

Treatment	Replicate	Determined [mg/kg dry weight]	Mean [mg/kg dry weight]	Expected [mg/kg] [1)]	% of expected	Mean [%]
T1	a	0.080	0.113	0.133	60.0	85.1
	b	0.119			89.4	
	c	0.138			103.5	
	d	0.117			87.6	
T2	a	0.209	0.234	0.267	78.3	87.7
	b	0.214			80.1	
	c	0.274			102.6	
	d	0.240			89.9	
T3	a	0.458	0.480	0.533	85.9	90.1
	b	0.451			84.6	
	c	0.524			98.3	
	d	0.489			91.8	

[1)] calculated for the 0-10 cm soil layer considering a soil density of 1.5 kg/dm³; calculation based on nominal content of a.i.

III. CONCLUSION

It can be concluded that after application of BAS 752 AM F tested at application rates of 2.0, 4.0 and 8.0 L BAS 752 AM F/ha no sustained adverse effects on earthworm populations occurred.

RMS Comments:
The outcome and utility of this study has been discussed in further detail in the earthworm risk assessment below (B.9.8.1).

Report: B.9.7.1/3
Schulz L., 2015b
Effects of BAS 750 01 F on earthworms under field conditions
2015/1000163

Guidelines: ISO 11268-3 (1999), Kula et al. (2006)-Technical Recommendations to ISO 11268-3 (1999)

GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F, batch no. FD-140113-0006, content of BAS 750 F: 98.9 g/L analysed

B. STUDY DESIGN

Test species:	Naturally occurring field population of earthworms comprising all mobile stages (juvenile and adult) of epigeic species such as *Lumbricus castaneus* and *Lumbricus spec.*, of endogeic species such as *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Aporrectodea spec*. as well as anecic species such as *Aporrectodea longa* and *Lumbricus terrestris.* All stages of earthworms were accounted for (juvenile, adult and indeterminable due to damage).
Test site:	Arable field site near Kettinge, Denmark with the total size of the test area being about 3100 m^2. The selected area did not receive an application of any crop protection product during the study apart from the test substance and the reference item, including the control plots. Application was onto bare soil and after 2 months sparing barley was seeded and then harvested 5 months post-application. Before use as a test site, the field was maintained according to general agricultural practice as a clover-grass mixture with no applied PPP for the preceding four years.
Test design:	Randomized block design, the study area was divided into 20 plots of 100 m^2 (10 m x 10 m) surrounded by 2m wide paths. 5 treatment groups (3 test substance treatment groups, control and a reference item) each with 4 replicates. Application was performed using a calibrated plot sprayer with a spray width of 2.5 m and with 10 TEEJET DG 80015 VS nozzles. Spray pressure was at 5 bar and maximum deviation was 5.20%. 12mm of precipitation on day 1 meant no irrigation was required.
Endpoints:	Total abundance and biomass of earthworms, species dominance.
Reference item:	Nutdazim 50 FLOW® (a.s.: carbendazim, 500 g/L nominal).
Test rates:	Treatment group 1: Untreated control (tap water); Treatment group 2: BAS 750 01 F applied at 3 L/ha; Treatment group 3: BAS 750 01 F applied at 6 L/ha; Treatment group 4: BAS 750 01 F applied at 12 L/ha; Treatment group 5: reference item applied at 20 L/ha (nominally equivalent to 10kg a.s./ha). All treatment groups were applied in 600 L water/ha.
Sampling method:	Surface monitoring occurred for all groups except the reference daily from day 1 until day 3 after application, by walking lengthwise through the centre of the plots and counting all visible earthworms along a 1 m wide stipe to both sides. There were two monitoring areas of 1m^2 per plot. Population sampling was performed in defined areas to assess earthworm populations 14 days before and three times after the application (1, 6 and 12 months after application). Earthworms were sampled from four 0.125 m^2 sampling areas per plot per sampling occasion exclusively from the central area (6m×6m). The sampling method was hand sorting combined with formalin extraction in the excavated hole. 2.5L of 0.2% formaldehyde solutions was uniformly poured into the holes for an extraction duration of at least 30 minutes. Adult earthworm were identified to the species level, and juveniles to the species level if possible, otherwise to the genus level.

Sampling dates: Pre-sampling: 24.03.2014 (2 weeks before application);
1st sampling: 05.05.2014 (about 1 month after application);
2nd sampling: 21.10.2014 (about 6 months after application);
3rd sampling: 14.04.2015 (about 12 months after application).

Test conditions: Natural field conditions; loamy sand (DIN 4220)/sandy loam (USDA). Application was performed on 07/07/2014, with low wind and no rain. Rainfall was recorded on day 1 after application (12.0mm).

pH	6.6,
WHC	27.0%,
C_{org}	0.99%,
Humus content (%):	1.70
A-horizon	10cm
Mean air temperatures during application:	12.5–19.9 °C,
Mean relative air humidity during application:	80%-95%.

Soil temperature during collection:
Pre-sampling: 8.7-9.7 °C, 1st sampling: 11.8-12.4 °C,
2nd sampling: 11.5-12.0 °C; 3rd sampling: 5.0–6.1 °C.

Moisture during collection:
Pre-sampling: 14.9%, 1st sampling: 11.2%,
2nd sampling: 16.8%; 3rd sampling: 14.4%.

Table B.9.7.1/3-1: Weather conditions during application and the days following application
(air temperature in 2 m height [°C], soil temperature in 10 cm depth [°C] and rainfall [mm])

Date	Air temperature [1] [°C]	Soil temperature [2] [°C]	Rainfall [2] [mm]	Irrigation [2] [mm]
07.04.2014 (application)	12.6	11.3	0.0	0.0
08.04.2014	10.5	11.0	12.0	0.0
09.04.2014	8.5	9.3	0.0	0.0
10.04.2014	7.0	8.9	0.0	0.0

1) recorded by weather station Abed 4920, Denmark
2) recorded on site

Statistics: ANOVA with treatment as fixed factor and block as random factor for pre-treatment sampling. Post-treatment sampling was analysed by a one-sided Dunnett-t-test for homogenous variances or one-sided Welch-t-test for inhomogeneous variances with Bonferroni-Holm adjustment. For the reference item treatment group to the control, t-tests for test substance data (one-sided smaller, $\alpha = 0.05$) and Student-t-test or Welch-t-test for reference item data (one-sided smaller, $\alpha = 0.05$). Statistical analyses were performed with ToxRat Professional 2.10.

II. RESULTS AND DISCUSSION

All the validity criteria were met for arable land field studies:

- ≥20 individuals per square metre (minimum 156.5 at pre-treatment sampling)
- Reasonable species diversity (8 species)
- ≥10% important species (65.8% abundance was *Aporrectodea caliginosa*)

The mean recovery of soil cores across all treatment groups and replicates was 123.6-151.1% of nominal concentrations. Spray target analyses indicated that for all replicates and treatment groups the mean recovery was 110.6-118.3%, confirming that the test substance was accurately applied. The limit of quantification was 0.01 mg/kg dry soil. The percentage of application volume actually applied of the intended volume was 93.53-109.87% in the control, 104.20-108.87% at 3L BAS 750 F/ha, 96.53-106.20% at 6L BAS 750 F/ha, 101.87-103.20% at 12L BAS 750 F/ha and 90.87-109.20% for the reference substance.

Surface monitoring on days 1-3 after application showed that there was no acute primary effect on earthworms by BAS 750 01 F. No alive, moribund or dead earthworms were found on the soil surface neither in the test substance nor in the control monitoring areas. Due to low soil moisture content as a result of deficit precipitation, for the first sampling period earthworms had retreated to lower soil layers and therefore only a portion of the earthworm population was assessable for this sampling.

No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rates of 3 and 6 L BAS 750 01 F at 1, 6 and 12 months after application. Furthermore, no statistically significant reductions in abundance and biomass of the dominant earthworm species (*Aporrectodea caliginosa*, *Lumbricus terrestris* and *Aporrectodea longa*) and ecological groups (endogeic and anecic earthworms) could be observed for the tested application rates of 3 and 6 L BAS 750 01 F about 1, 6 and 12 months after application. The only significant effect of the test substance was at 12L BAS 750 01 F/ a after 12 months.

No statistically significant reductions in total earthworm abundance and biomass, in abundance and biomass of the dominant earthworm species (*Aporrectodea caliginosa*, *Lumbricus terrestris* and *Aporrectodea longa*) and in abundance and biomass of the dominant ecological groups (endogeic and anecic earthworms) could be observed for the tested application rate of 12 L BAS 750 01 F at 1 and 6 months after application. After 12 months, deviations from control could be observed for total anecic adult abundance and biomass (total, total adult, total anecic and total anecic adult biomass) in the highest treatment group of 12 L BAS 750 01 F/ha about 12 months after application. This difference can be mainly attributed to the earthworm species *Lumbricus terrestris*. For abundance, the deviation to the control was -43.9%, for biomass the deviation to the control was -61.7%. It is reported that total abundance and biomass of *Lumbricus terrestris* were already reduced in the control at pre-sampling (deviation from control in abundance: -37.5 %, in biomass: -54.5 %), and that the observed reductions in abundance and biomass at the tested application rate of 12 L BAS 750 01 F are and considered as not test substance related.

The concentration of the reference item was not sufficient to cause significantly detectable effects of the positive control . Abundance of the test organisms should ideally be reduced by 40-80%, although at no sampling period was the total mean abundance significantly less than the control. However there was a significant reduction in total biomass in the first and third sampling periods.

None of the application rates tested up to a rate of 12 L BAS 750 01 F/ha caused statistically significant effects on total juvenile abundance and biomass about 1, 6 and 12 months after application.

The results are summarised in Table B.9.7.1/3-2.

Table B.9.7.1/3-2: Summary of total earthworm abundance and biomass in a field study with BAS 750 01 F

Treatment	Pre-sampling 24.03.2014 [1)]	First sampling 05.05.2014	Second sampling 21.10.2014	Third sampling 14.04.2015
Total earthworm abundance [individuals/m²]				
Control	169.5 ± 58.27	50.0 ± 25.51	145.5 ± 14.08	161.5 ± 25.27
3 L BAS 750 01 F/ha	166.5 ± 60.87	56.0 ± 26.08	220.5 ± 84.37	157.0 ± 17.93
% of control	**98.2**	**112.0**	**151.6**	**97.2**
6 L BAS 750 01 F/ha	166.5 ± 67.08	42.5 ± 21.32	192.5 ± 53.97	164.0 ± 27.37
% of control	**98.2**	**85.0**	**132.3**	**101.6**
12 L BAS 750 01 F/ha	166.0 ± 73.41	53.0 ± 11.19	153.0 ± 73.4	126.5 ± 54.66
% of control	**97.9**	**106.0**	**105.2**	**78.3**
Reference	156.5 ± 88.41	28.0 ± 9.38*	165.5 ± 35.38	131.0 ± 26.96
% of control	**92.3**	**56.0**	**113.8**	**81.8**
Total earthworm biomass [g/m²]				
Control	139.89 ± 30.31	44.42 ± 21.57	90.51 ± 21.21	134.98 ± 19.54
3 L BAS 750 01 F/ha	131.26 ± 35.37	52.19 ± 5.59	158.05 ± 87.42	116.41 ± 24.47
% of control	**93.8**	**117.5**	**174.6**	**86.2**
6 L BAS 750 01 F/ha	140.88 ± 30.78	46.86 ± 24.85	162.23 ± 47.02	130.18 ± 18.91
% of control	**100.7**	**105.5**	**179.2**	**96.5**
12 L BAS 750 01 F/ha	126.82 ± 56.31	39.96 ± 9.91	104.13 ± 54.61	82.37 ± 35.54 *
% of control	**90.7**	**90.0**	**115.1**	**61.0 ***
Reference	136.02 ± 67.41	22.09 ± 5.14*	102.23 ± 17.65	90.70 ± 2.05*
% of control	**97.2**	**49.7**	**113.0**	**67.2**

[1)] Prior to application.

* Statistically significant differences compared to the control (Dunnett t-test, $\alpha = 0.05$).

The earthworm abundance and biomass in the reference item was statistically significantly reduced by 44.0% and 50.3% at the 1st sampling, respectively (Student t-test, $\alpha = 0.05$), fulfilling the guideline recommendation for this sampling period only.

Table B.9.7.1/3-3: Summary of abundance and biomass for the three most dominant earthworm species

Sampling date		pre-sampling	1st sampling	2nd sampling	3rd sampling
Treatment			**Total *A. longa* abundance (ind./m²)**		
Control	ind./m²	29.5	6.5	43.0	46.0
	%	**100.0**	**100.0**	**100.0**	**100.0**
Test item (3 L BAS 750 01 F/ha)	ind./m²	35.0	7.0	71.5	49.0
	%	**118.6**	**107.7**	**166.3**	**106.5**
Test item (6 L BAS 750 01 F/ha)	ind./m²	24.5	4.5	48.5	47.5
	%	**83.1**	**69.2**	**112.8**	**103.3**
Test item (12 L BAS 750 01 F/ha)	ind./m²	29.5	3.5	45.5	38.0
	%	**100.0**	**53.8**	**105.8**	**82.6**
Reference (20 L/ha)	ind./m²	37.0	7.5	52.0	44.0
	%	**125.4**	**115.4**	**120.9**	**95.7**
Treatment			**Total *A. longa* biomass (g/m²)**		
Control	ind./m²	42.54	5.54	30.06	40.25
	%	**100.0**	**100.0**	**100.0**	**100.0**
Test item (3 L BAS 750 01 F/ha)	ind./m²	50.17	7.47	62.05	38.12
	%	**117.9**	**134.8**	**206.4**	**94.7**
Test item (6 L BAS 750 01 F/ha)	ind./m²	32.08	4.55	51.35	44.95
	%	**75.4**	**82.1**	**170.8**	**111.7**
Test item (12 L BAS 750 01 F/ha)	ind./m²	34.05	3.59	34.04	31.98
	%	**80.0**	**64.7**	**113.3**	**79.5**
Reference (20 L/ha)	ind./m²	48.02	9.51	37.83	31.17
	%	**112.9**	**171.6**	**125.8**	**77.4**
Treatment			**Total *L. terrestris* abundance (ind./m²)**		
Control	ind./m²	12.0	7.5	29.0	28.5
	%	**100.0**	**100.0**	**100.0**	**100.0**
Test item (3 L BAS 750 01 F/ha)	ind./m²	15.0	8.0	33.5	26.0
	%	**125.0**	**106.7**	**115.5**	**91.2**
Test item (6 L BAS 750 01 F/ha)	ind./m²	15.5	10.5	31.0	26.0
	%	**129.2**	**140.0**	**106.9**	**91.2**
Test item (12 L BAS 750 01 F/ha)	ind./m²	7.5	9.0	24.0	16.0 *
	%	**62.5**	**120.0**	**82.8**	**56.1**
Reference (20 L/ha)	ind./m²	15.5	3.0	23.5	22.0
	%	**129.2**	**40.0**	**81.0**	**77.2**
Treatment			**Total *L. terrestris* biomass (g/m²)**		
Control	ind./m²	15.88	21.25	27.84	57.91
	%	**100.0**	**100.0**	**100.0**	**100.0**
Test item (3 L BAS 750 01 F/ha)	ind./m²	11.37	23.49	38.11	45.37
	%	**71.6**	**110.6**	**136.9**	**78.3**
Test item (6 L BAS 750 01 F/ha)	ind./m²	29.72	27.07	56.64	45.68
	%	**187.2**	**127.4**	**203.4**	**78.9**
Test item (12 L BAS 750 01 F/ha)	ind./m²	7.23	14.74	28.62	22.20 *
	%	**45.5**	**69.4**	**102.8**	**38.3**
Reference (20 L/ha)	ind./m²	22.21	3.63	22.27	31.57 *
	%	**139.9**	**17.1**	**80.0**	**54.5**

Treatment		Total *A. caliginosa* abundance (ind./m²)			
Control	ind./m²	111.5	30.5	65.5	80.5
	%	**100.0**	**100.0**	**100.0**	**100.0**
Test item (3 L BAS 750 01 F/ha)	ind./m²	102.0	33.0	99.5	73.0
	%	**91.5**	**108.2**	**151.9**	**90.7**
Test item (6 L BAS 750 01 F/ha)	ind./m²	108.0	23.5	103.0	82.5
	%	**96.9**	**77.1**	**157.3**	**102.5**
Test item (12 L BAS 750 01 F/ha)	ind./m²	114.0	37.0	75.5	63.0
	%	**102.2**	**121.3**	**115.3**	**78.3**
Reference (20 L/ha)	ind./m²	91.5	17.0 *	82.5	56.0
	%	**82.1**	**55.7**	**126.0**	**69.6**
Treatment		**Total *A. caliginosa* biomass (g/m²)**			
Control	ind./m²	77.92	16.13	31.15	35.08
	%	**100.0**	**100.0**	**100.0**	**100.0**
Test item (3 L BAS 750 01 F/ha)	ind./m²	66.38	19.49	53.46	30.80
	%	**85.2**	**120.8**	**171.6**	**87.8**
Test item (6 L BAS 750 01 F/ha)	ind./m²	74.34	14.32	51.42	37.62
	%	**95.4**	**88.8**	**165.1**	**107.2**
Test item (12 L BAS 750 01 F/ha)	ind./m²	81.33	20.69	39.76	26.21
	%	**104.4**	**128.3**	**127.6**	**74.7**
Reference (20 L/ha)	ind./m²	62.35	8.79 *	40.00	25.49
	%	**80.0**	**54.5**	**128.4**	**72.7**

III. CONCLUSION

It can be concluded that the application of BAS 750 01 F tested at application rates of 3 , 6 and 12 L BAS 750 01 F/ha had no adverse effects on single species, ecological groups and total earthworm abundance and biomass about one year after application.

RMS Comment: The outcome and utility of this study has been discussed in further detail in the earthworm risk assessment below (B.9.8.1).

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

Report: B.9.7.2/1
Friedrich S., 2015a
Effects of BAS 750 01 F on the reproduction of the collembolan *Folsomia candida*
2015/1000885
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species:	Collembola (*Folsomia candida*), from in-house culture, juveniles and adults, 9-12 days old.
Test design:	28-d exposure in treated artificial soil; different concentrations of the test substance are mixed homogeneously into the soil which is filled in glass containers before the Collembola are introduced on top of the soil; 6 treatment groups (5 test substance concentrations, control); 4 replicates in the test substance treatment group, 8 replicates in the control group, each with 10 Collembola; assessment of mortality, reproduction and behavioural effects after 28 days. The adults and juveniles were extracted by floatation in ink-darkened water. Counting was carried out using an automated cell counting technique (LemnaTec Scanalyzer; average error <10 %).
Endpoints:	Mortality and reproduction parameters.
Reference item:	Boric acid (100% analysed), the effects of the reference item were investigated in a separate study (July 2014). The EC_{50} (reproduction) was determined to be 104 mg/kg dsw, which is close to the guideline recommended concentration of 100 mg/kg dsw, therefore the sensitivity of the test system was confirmed.
Test rates:	Control (deionised water), 84.6, 110.0, 143.0, 185.9 and 241.7 mg BAS 750 01 F/kg dry soil. A stock solution of the test substance was prepared, and was stepwise diluted with deionised water such that each solution contained the amount of test substance in 25 mL required to dose 250 g soil (dry weight, dsw). The test solutions were mixed with their corresponding artificial soil allocation using a laboratory mixer.
Test conditions:	Artificial soil according to OECD 232 (5% sphagnum peat, 20 % Kaolin clay, 74.7 % quartz sand, 0.3 % $CaCO_3$, mixed with a laboratory mixer and, post treatment, allocated in 30 g (wet weight) portions per replicate test unit (150 mL glass container); pH at test initiation 6.05 - 6.12, at test termination 5.79 – 5.84; water content at test initiation 57.9% - 58.1% of maximum water holding capacity (WHC); 56.5% - 57.4% of maximum WHC at test termination; temperature 18.2°C - 22°C; photoperiod: 16 hours light: 8 hours dark; light intensity: 530 lux. Food – 2 mg dried yeast at the start of the test and after 14 days. The test vessels were opened twice a week for aeration.
Statistics:	Mortality calculated (%), missing adults counted as dead. Reproductive output (%) was calculated and compared to the control. Fisher's Exact Binomial with Boneferroni Correction for mortality (α = 0.05, one-sided greater), Dunnett t-test test for reproduction (α = 0.05, one-sided smaller). Statistical software used was ToxRat Professional 3.0.beta (2014).

II. RESULTS AND DISCUSSION

BAS 750 01 F caused no statistically significant effects on survival to *Folsomia candida* at concentrations up to and including 241.7 mg/kg dry soil, the highest concentration tested (Fisher's Exact Binomial Test with Bonferroni correction, α = 0.05, one-sided greater). Mortality in the test substance treatments ranged from 2.5% to 7.5%, compared to 5.0% in the control.

The reproduction in the control reached a mean of 864 juveniles. Reproduction rates in 84.6, 110.0, 143.0, 185.9 and 241.7 mg test substance/kg dry soil were 866, 902, 886, 814 and 749 juveniles, respectively. No statistically significant reduction was observed at any concentration tested (Dunnett t-test, $\alpha = 0.05$, one-sided smaller). The test was not designed to obtain an EC_x. Also, the test showed no significant, slight effects at the highest test rate. Hence a proper derivation of the EC_x values is not possible and no further EC_x calculations were performed. The results are summarised in Table B.9.4.2.1-1.

Table B.9.7.2/1-1: Effects of BAS 750 01 F on *Folsomia candida* in a 28-day reproduction study

BAS 750 01 F [mg/kg dry soil]	**Control**	**84.6**	**110.0**	**143.0**	**185.9**	**241.7**
Mortality (day 28) [%]	5.0	5.0	2.5	7.5	5.0	2.5
Mean no. of juveniles (day 28)	864	866	902	886	814	749
Coefficient of Variation (Reproduction; %)	13.3	17.8	8.1	3.3	18.0	22.3
Reproduction (day 28) [% of control]	100	100	104	103	94	87
Endpoints [mg BAS 750 01 F/kg dry soil]						
NOEC (mortality/reproduction)	≥ 241.7					
LC_{50} (mortality)	> 241.7					
EC_{50} (reproduction)	> 241.7					

Validity Criteria

The study meets the validity criteria for the control test group as specified in OECD 232:

- Mean adult mortality did not exceed 20 % (being 5 %)
- Mean number of juveniles per vessel was at least 100 (being 864)
- Coefficient of variation (number of juveniles) was less than 30 % (being 13.3 %)

III. CONCLUSION

In a 28-day reproduction study on Collembola (*Folsomia candida*) with BAS 750 01 F the LC_{50} was > 241.7 mg BAS 750 01 F/kg dry soil. The overall NOEC was ≥ 241.7 mg BAS 750 01 F/kg dry soil.

RMS Comments

The test was carried out according to GLP and follows guidelines OECD 232 and ISO 11267 with no significant deviations noted. The study had a 16 h:8 h light/dark ratio, whilst the guideline states that preferably a 12 h:12 h light/dark regime should be implemented. The light:dark ratio effects the activity of the test organisms, potentially affecting the level of exposure. No explanation is provided in the full study report as to why they have changed this parameter. However, given the apparent low toxicity of the test substance, this deviation is not considered to have had a significant effect on the outcome of the study.

The results for reproduction suggest a potential dose response, with 6 % and 13 % reduction of reproduction compared to the control in the 185.9 mg/kg and 241.7 mg/kg treatment groups, respectively. In the case of the latter the coefficient of variation is the highest amongst the treatment groups, being 22.3 %. The table below contains the raw data from the study:

Treatment group	mg test item/kg soil d.w.					
	Control	84.6	110.0	143.0	185.9	241.7
Replicate	Number of juveniles per replicate (4 weeks after test initiation)					
1	680	891	900	909	998	808
2	935	935	971	912	803	671
3	863	994	937	852	639	951
4	813	644	801	871	815	566
5	796					
6	1057					
7	945					
8	819					
Mean	**864**	**866**	**902**	**886**	**814**	**749**
±SD	114.6	153.9	73.5	29.4	146.7	167.2
cv%	13.3	17.8	8.1	3.3	18.0	22.3
Reproduction (% of control)	**100**	**100**	**104**	**103**	**94**	**87**

Not statistically significantly different compared to control (Dunnett-t-test for reproduction; α = 0.05, one-sided smaller)

SD: standard deviation, cv %: coefficient of variation, d.w.: dry weight (of artificial soil)

Apart from one replicate (566), the range of results (671-951) in the 241.7 mg/kg treatment group is similar to that of the control (680-1057). This indicates that the low result in the former is due to a single poor performing replicate rather than an overall impact of the treatment – if it was treatment-related, a reduction in all replicates would be expected. Therefore, in the absence of more data, the RMS considers the endpoint derived from the study to be appropriate.

The agreed endpoint considered suitable for use in the risk assessment is:
28 day NOEC ≥ 241.7 mg BAS 750 01 F/kg dsw (24.07 mg a.s./kg dsw)

Report:	B.9.7.2/2 Ganβmann M., 2015b Effects of BAS 750 01 F on reproduction of the predatory mite *Hyposaspis aculeifer* in artificial soil with 5% peat 2014/1242737
Report:	B.9.7.2/2a Ganβmann M., 2015c 1st Final report amendment: Effects of BAS 750 01 F on reproduction of the predatory mite *Hyposaspis aculeifer* in artificial soil with 5% peat 2015/1035024
Guidelines:	OECD 226 (2008)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test species: Soil mites: *Hypoaspis aculeifer* (CANESTRINI); age: approximately 11 days after reaching the adult stage; source: in-house culture.

Test design: The effects of BAS 750 01 F on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226. Different concentrations of the test substance were homogeneously mixed into the artificial soil (5% peat) which was then filled into glass vessels after which the soil mites were introduced on top of the soil; 6 treatment groups (5 test substance concentrations, control); 8 replicates/control group and 4 replicates/test substance treatment group each with 10 female soil mites. Assessment of adult mortality and reproduction effects was carried out after 14 days. The mites were extracted using a Kempson Extractor (extraction efficiency reported to be 96.1 %), and collected in a fixing liquid before counting manually, using a binocular microscope for the juveniles.

Endpoints: Mortality and reproduction rate (no. juveniles) after 14 days.

Reference item: Perfekthion (content of a.s. dimethoate: 400 g/L nominal). The effects of the reference item were investigated in a separate study (June 2014). The study determined an EC_{50} (reproduction) of 5.5 mg a.s./kg dsw, which falls within the range described in OECD 226 (3-7 mg a.s./kg dsw).

Test concentrations: Control (deionised water), 53.3, 80.0, 120, 180 and 270 mg BAS 750 01 F/kg dry soil. The lower test substance concentrations were prepared by step-wise dilution of a stock solution of the highest test concentration. 26.9 g of each solution was added to the corresponding

allocation of artificial soil (200 g dry weight) and mixed with a laboratory mixer. There were no significant deviations from the nominal concentrations (< 5 %).

Test conditions: Artificial soil according to OECD 226 (5 % peat, 20 % Kaolin clay, 74.8 % quartz sand, 0.2 % $CaCO_3$, after treatment the soil was allocated to 100 mL glass containers in 20 g (dry weight) portions per replicate). pH 6.0 - pH 6.1 at test initiation, pH 5.8 - pH 6.1 at test termination; water content at test initiation 50.1% - 52.4% of maximum water holding capacity (WHC) and 46.6% - 48.7% of maximum WHC at test termination; temperature 18 °C – 22 °C; photoperiod: 16 h light: 8 h dark; light intensity: 400 - 800 lux. Feeding of mites with *Tyrophagus putrescentiae* every 2-3 days, alongside aeration of the test vessels.

Statistics: Mortality data (dead + missing mites) in the treated groups was compared with the control using Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$, one-sided greater). Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$), respectively. Williams-t-test was used to compare the reproductive output of the treated groups with that of the control group ($\alpha = 0.05$, one-sided smaller), Probit Analysis for EC-values. Statistical software used was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

Mortality rates of adult soil mites of 3.0% - 8.0% were recorded in the test substance treatment groups, compared to 0.0% mortality in the control group. The observed mortality rates in the test substance treatment groups compared to control were not statistically significant

The mean reproduction in the control reached 277 juveniles. The rates of reproduction in the test substance treated groups were not statistically significantly different compared to those in the control up to and including 180 mg BAS 750 01 F/kg dry soil. At the highest concentration of 270 mg test substance/kg dry soil, the reproduction rate was statistically significantly reduced.

No behavioural abnormalities were observed in any of the treatment groups. The EC_{10} was determined to be 187.7 mg BAS 750 01 F/kg dry soil. The results are summarised in Table B.9.7.2/2-1.

Table B.9.7.2/2-1: Effects of BAS 750 01 F on *Hypoaspis aculeifer* in a 14-day reproduction study

BAS 750 01 F [mg/kg dry soil]	**Control**	**53.3**	**80.0**	**120**	**180**	**270**
Mortality (day 14) [%]	0	3	3	8	3	8
Mean No. of juveniles (day 14)	277	276	292	242	274	219
Coefficient of variation (Reproduction; %)	5.4	6.5	11.9	4.1	6.2	12.8
Reproduction (day 14) [% of control]	--	100	105	87	99	79 *
Endpoints [mg BAS 750 01 F/kg dry soil]						
$NOEC_{mortality}$	≥ 270					
$NOEC_{reproduction}$	180					
EC_{10}	187.7					
EC_{50}	> 270					
LC_{50}	> 270					

* Statistically significantly different compared to the control (William's t-test, α = 0.05, one-sided smaller).

Validity Criteria
The study meets the validity criteria for the control specified in OECD 226:

- Mean adult female mortality was not greater than 20 % (being 0 %)
- Mean number of juveniles per replicate was at least 50 (being 249)
- Coefficient of variation calculated for the number of juvenile mites per replicate was not greater than 30 % at the end of the definitive test (being 5.4 %)

III. CONCLUSION

In a 14-day reproduction study with BAS 750 01 F on soil mites (*Hypoaspis aculeifer*), the LC_{50} was estimated to be > 270 mg BAS 750 01 F/kg dry soil. The NOEC for mortality was determined to be ≥ 270 mg BAS 750 01 F/kg dry soil. The NOEC for reproduction was determined to be 180 mg BAS 750 01 F/kg dry soil and the EC_{10} was determined to be 187.7 mg BAS 750 01 F/kg dry soil.

RMS Comments
The study was carried out according to GLP and follows guideline OECD 226 with no deviations noted.

The agreed endpoint considered suitable for use in the risk assessment is:
14 day NOEC (reproduction) = 180 mg BAS 750 01 F/kg dsw (17.92 mg a.s./kg dsw)

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

BAS 750 01 F is the representative formulation for the approval of the new fungicidal active substance BAS 750 F. BAS 750 01 F is an EC (emulsifiable concentrate) formulation, containing 100 g BAS 750 F/L intended for use in winter and spring cereals.

Exposure

Table B.9.8-1 Proposed use pattern of BAS 750 01 F

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				BAS 750 F [g a.s./ha]	BAS 750 01 F [L/ha]
cereals	30 - 69	2	14	150	1.5

The exposure to soil organisms was estimated by calculating the maximum predicted environmental concentrations in soil (PEC_{soil}). For multiple applications, the worst-case maximum PEC_{soil} will be the one immediately after the final application. Regarding the PEC_{soil}, the worst-case use pattern of BAS 750 01 F envisages two applications to cereals with a maximum single dose rate of 1.5 L/ha, corresponding to 150 g BAS 750 F/ha. Since the worst-case DT_{90} values of BAS 750 F and 1,2,4-triazole exceed 365 days, $PEC_{soil, accu}$ values were calculated, assuming accumulation concentration and reflecting multi-year use. For details, see chapter III CP B.8.1. The resulting maximum PEC_{soil} values are presented in the table below

Table B.9.8-2 Worst-case PEC_{soil} values for BAS 750 F

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
BAS 750 F	0.080	0.308*
1,2,4-triazole	0.001	0.001*

*PEC values are used for TER calculations.

B.9.8.1 Earthworms

For the risk assessment on earthworms, chronic studies on earthworms have been carried out with BAS 750 F, the metabolite 1,2,4-triazole and with the formulation BAS 750 01 F (see table B.9.8.1-1). Additionally, and to further strengthen the risk assessment, two earthworm field effect studies were carried out with the representative formulation BAS 750 01 F and a different mixed-active formulation, BAS 752 AM F (containing 100.0 g BAS 750 F/L and 50 g fluxapyroxad/L; please see III CP B.9.7.1 for details), respectively.

Further details on the studies with the formulation are given above (III CP B.9.7). Details on the chronic studies with the active substance and the metabolite are presented in III CA B-9 and the relevant EU documents. The earthworm endpoints are summarised in the table below.

For substances with log P_{ow} values > 2 and studies with a high content of organic material in the artificial soil (i.e. 10% peat), the resulting endpoints have to be corrected by a factor of 2 (f_{oc}) in the risk assessment in order to address lower contents of organic material in natural soil.
The log P_{ow} for the active substance BAS 750 F is > 2 (i.e. 3.4). In an expert meeting (PRAPeR meeting 133, 2015) it was the opinion of EFSA, that for substances with a log K_{OW} >2, the correction factor should be applied to toxicity endpoints:

'It is proposed to reconfirm that the correction factor of 2 is applied even if a lower organic matter content was used in the test (i.e 5%) until better guidance is available".

"It was noted that the application of the correction factor of 2 may not be totally appropriate for collembolan and soil mites studies where 5% OM is udes in the test guideline. However, in the absence of any better data, it was agreed that the factor of 2 should be applied. It was also mentioned that agricultural soils in the EU normally have an OM content below 5%."

Commission Regulation (EU) 283/2013 and 284/2013 require estimates of EC_x (e.g. EC_{10}, EC_{20}) together with the NOEC. The chronic earthworm studies were designed for deriving a NOEC value with 5 dose rates (instead of 8 proposed for the EC_x design). In addition, the effect levels were rather low ($NOEC_{corr}$ = ≥3.98 mg a.s./kg dsw) and/or no clear dose response has been observed in the chronic earthworm studies. In this case, the NOEC should be retained as the primary endpoint as a proper derivation of an EC_{10} value is not possible. In the study with the BAS 750 F technical, EC_{10} values were calculated, and as they were lower than the NOEC, have been considered as more appropriate for the risk assessment.

Table B.9.8.1-1 Summary of earthworm endpoints for BAS 750 01 F, BAS 750 F and 1,2,4-triazole

Test substance	Test species	Endpoint	Value [mg/kg dry soil]	Reference
Acute toxicity [1)]				
BAS 750 F [#]	*Eisenia fetida*	$LC_{50\ CORR}$	> 500	B.9.4.1/1 Friedrich S., 2015a
BAS 750 F in BAS 750 01 F [##]		$LC_{50\ CORR}$	>24.9	B.9.7.1/1 Ganβmann M., 2015a
Chronic toxicity				
BAS 750 F [###]	*Eisenia fetida*	EC_{10CORR}	2.65 (1.63-4.36) [2)]	B.9.4.1/2 Friedrich S., 2013a
1,2,4-triazole [*]		NOEC	1.0	EFSA Journal 2014; 12(1): 3485
BAS 750 F in BAS 750 01 F		$NOEC_{CORR}$	≥3.98 [2) 3)]	B.9.7.1/1 Ganβmann M., 2015a
Field studies				
BAS 752 AM F [4)]	earthworm field population	See field studies section below		B.9.7.1/2 Hamberger A., 2015a
BAS 750 01 F	earthworm field population	See field studies section below		B.9.7.1/3 Schulz L., 2015b

1) Acute studies are listed for reference, but not used in the following risk assessment, as acute studies are no longer required according to EU Commission Regulation No.283/2013.

2) Toxicity endpoint is re-adjusted by a soil factor of 2 to address the organic content of the soil (peat 10%), since the log P_{ow} for the active substance BAS 750 F is > 2 (3.4).

3) Endpoint expressed in terms of active substance – please see study evaluation in section III CP B.9.7.1/1.

[4)] Study was performed with BAS 752 AM F, containing 100.0 g BAS 750 F/L and 50 g BAS 700 F (fluxapyroxad)/L (please see III CP B.9.7.1/2).
[#] Study is presented as additional information in Volume III CA Section B.9.7.1.
[##] Study is presented as additional information in Section B.9.7.1/1
[###] Study is presented in chapter Volume III CA Section B.9.4.1.
[*] EU agreed, e.g. within renewal of active substance Tebuconazole: EFSA Journal 2014; 12(1):3485; 88 pp.

Risk assessment for earthworms

Although acute toxicity data is provided, acute studies are no longer a requirement for the risk assessment to earthworms under EU Commission Regulation 283/2013. Therefore this data provides additional information only.

The potential long-term risk of BAS 750 01 F to earthworms was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum PEC_{soil} values using the following equation:

$$TER_{LT} = \frac{NOEC\,[mg/kg\ dry\ soil]}{PEC_{soil}\,[mg/kg\ dry\ soil]}$$

The resulting TER_{LT} values are presented below:

Long-term TER values for earthworms

Test substance	NOEC [mg/kg dry soil]	PEC_{soil} [mg/kg dry soil]	TER_{LT}	TER trigger
BAS 750 F	(EC_{10}) 2.65 [1)]	0.308	8.6	5
1,2,4-triazole	1.0	0.001	1000	
BAS 750 F in BAS 750 01 F	≥ 3.98 [1)]	0.308	≥ 12.92	

[1)] Toxicity endpoint is adjusted by a soil factor of 2 to address the organic content of the soil, since the log P_{ow} for the substance is > 2.

The long-term TER values for BAS 750 F, 1,2,4-triazole and BAS 750 F as contained in BAS 750 01 F are above the Commission Regulation (EU) 546/2011 trigger of 5. Therefore, chronic risk for earthworms arising from exposure to BAS 750 F in BAS 750 01 F is acceptable.

Field studies

The chronic risk assessment indicates an acceptable risk; therefore no further data are required. Nevertheless, since these additional studies have been provided, they have been evaluated to confirm a low risk. The results of the studies are summarised in III CP B.9.7.1. An in-depth evaluation of the studies and their impact on the risk assessment follows:

Schulz L., 2015b

The study with BAS 750 01 F was conducted according to the principles of GLP and follows the guidance available (ISO 11268-3, 1999, Kula et al. 2006) with a number of deviations noted.

The method of analysis for BAS 750 F was marginally over the recommended range of 50-150% in Kula et al. (2006) in the fortification level of 0.200 mg/kg being 151.08%. The recoveries at 0.400 mg/kg and 0.800 mg/kg are lower 126.75% and 123.61% respectively and the recoveries in spray targets and desorption experiments are acceptable.

In the reference item treatment, abundance was reduced for the first sampling by 44.0%, but by 18.9% in the third sampling. For the second sampling, there was no significant reduction in abundance. Overall, the results of the reference cast doubt on the reliability of the study and the method used for this test to be sensitive enough to detect negative effects that the test substance had upon the test organisms.

No extraction efficiency assays were conducted, although these are not required where a combination of hand-sorting and an extraction method is used as per Kula et al. 2006.

The soil profile for the field and the sunshine duration was not presented in the study report, and whether the taxonomy of the test organisms was determined on site or post-sampling with fixing was not mentioned, although it was said to have occurred within 48 hours of sampling. As all the validity criteria were met and no negative effects were observed in the controls, these climatic and taxonomic omissions are not expected to have adversely affected the study.

The study location was Kettinge, Denmark, part of the Northern zone. Although the climate conditions are comparable to those of the UK, other member states will need to consider the representativeness of the study location.

Hamberger A., 2015a

The study with BAS 752 AM F was conducted according to the principles of GLP and follows the guidance available (ISO 11268-3, 1999, Kula et al. 2006) with a number of deviations noted.
No extraction efficiency assays were conducted, although these are not required where a combination of hand-sorting and an extraction method is used as per Kula et al. 2006. In addition, no environmental condition measurements were taken prior to application. However, the timing of application fits with the timing of BBCH 30 (earliest application according to GAP is BBCH 30), so the conditions are thought to be representative. Large standard deviations for total earthworm abundance were noted in the controls, which limits the ability of the study to detect statistically significant differences. This may affect the results of the study, as a notable difference (not calculated to be statistically significant) between the control population of *Aporrectodea caliginosa* adults and those treated with 8.0 L f.p./ha was spotted at the fourth sampling occasion (Table B.9.7.1/1-2).
Also, the study was conducted with a different product to the representative formulation (BAS752 AM F, a mixed active formulation, instead of BAS 750 F). This formulation consists of 50 g/L Fluxapyroxad and 100 g/L BAS 750 F. According to the EFSA conclusion of Fluxapyroxad, the chronic toxicity to Earthworms is 21.3 mg a.s./kg dsw (56 d NOEC). This contrasts with the chronic toxicity of BAS 750 F which is 7.9 mg a.s./kg (56 d NOEC). Although BAS 750 F appears to be the more toxic active substance to earthworms (and is present at higher concentration than Fluxapyroxad) the addition of another active substance adds an element of uncertainty to any potential effects observed, as these could be due to the combination of actives instead of BAS 750 F alone. This leads the RMS to question the utility of this study for the review of BAS 750 F. That said, no statistically significant adverse effects were noted on the individual species analysed, as well as overall abundance/biomass, or adult:juvenile ratio, at any test rate, therefore the study does not appear to cast doubt onto the conclusions of the first tier risk assessment.

Conclusion

Overall, whilst neither of the field studies submitted is ideal due to the deviations outlined above, they do not indicate that the use of either product containing BAS 750 F results in effects on earthworm populations that would undermine the first-tier risk assessment presented earlier in the document. Given the acceptability of the first-tier risk assessment and the above conclusion no further consideration of the risk to earthworms is required.

In conclusion, an acceptable risk to earthworms can be concluded for the proposed uses of BAS 750 F.

B.9.8.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

According to the Commission Regulation (EU) No 283/2013, in accordance with Regulation No. 1107/2009, testing of sub-lethal effects on Collembola and soil mites is required if the standard HQ for arthropods (*Typhlodromus pyri* and *Aphidius rhopalosiphi*) is above 2.

Toxicity

The calculated $HQ_{in\text{-}field}$ for arthropods (*T. pyri* and *A. rhopalosiphi*) are above 2 (please refer to chapter Vol III CP B.9.6). Thus, testing of potential sub-lethal effects on soil macro-organisms other than earthworms was triggered (Commission Regulation (EU) 283-2013). Chronic toxicity studies with BAS 750 F and BAS 750 01 F have been carried out with *Folsomia candida* and *Hypoaspis aculeifer* (see III CA Section B.9.4.2 and III CP Section B.9.7.2).

Table B.9.8.2-1: Summary of chronic endpoints on other soil macro-organism for BAS 750 01 F

Test substance	Test species	Endpoint	Value [mg/kg dry soil]	Reference
BAS 750 F [#]	*Folsomia candida*	$NOEC_{corr}$	≥ 200	B.9.4.2/1 Friedrich S., 2013b
1,2,4-triazole [*]			1.8	EFSA Scientific Report 138, 2008
BAS 750 F in BAS 750 01 F			> 12.035 [1)]	B.9.7.2/1 Friedrich S., 2015a
BAS 750 F [#]	*Hypoaspis aculeifer*		≥ 500	B.9.4.2/2 Schulz L., 2014a
1,2,4-triazole [#]			171	B.9.4.2/3 Schulz L., 2014b
BAS 750 F in BAS 750 01 F			8.96 [1)]	B.9.7.2/2 Ganβmann M., 2015b

[#] Study is presented in III CA B.9.4.2
[1)] Taking into account a density of BAS 750 01 F of 0.993 g/cm^3.

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

For risk assessment, the chronic toxicity data (NOEC for reproduction) are compared with the highest available predicted environmental concentration, in this case the PECsoil (Accumulation). The results are presented in Table B.9.8.1-2

For substances with log P_{ow} values > 2 and studies with a high content of organic material in the artificial soil (i.e. 10% peat), the resulting endpoints have to be corrected by a factor of 2 (f_{oc}) in the risk assessment.
The log P_{ow} for the active substance BAS 750 F is > 2 (i.e. 3.4). In an expert meeting (PRAPeR meeting 133, 2015) it was the opinion of EFSA, that for substances with a log K_{OW} >2, the correction

factor should be applied to toxicity endpoints unless it has been demonstrated that lowering the organic carbon content allows for the correction factor to not be used.

Commission Regulation (EU) 283/2013 and 284/2013 require estimates of EC_x (e.g. EC_{10}, EC_{20}) together with the NOEC. Both springtail and soil mite studies were designed for deriving a NOEC. The effect levels in both studies were rather low (see table B.9.8.1-2) and/or no clear dose response has been observed. In this case, the NOEC should be retained as the primary endpoint as a proper derivation of an EC_{10} value is not possible. Hence, the NOEC was used in the risk assessment below.

Table B.9.8.2-2: Long-term TER values for soil macro organisms

Test substance	Test species	$NOEC_{corr}$ [mg/kg dry soil]	PEC_{soil} (ACCUMULATION) [mg/kg dry soil]	TER_{LT}	TER trigger
BAS 750 F [#]	*Folsomia candida*	≥ 200	0.308	≥ 649	5
1,2,4-triazole [*]		1.8	0.001	1800	
BAS 750 F in BAS 750 01 F		> 12.035	0.308	> 39	
BAS 750 F [#]	*Hypoaspis aculeifer*	≥ 500	0.308	≥ 1623	
1,2,4-triazole [#]		171	0.001	171000	
BAS 750 F in BAS 750 01 F		8.96	0.308	29.1	

[#] Study is presented in chapter III CA B.9.4.
[*] EU agreed, e.g. within evaluation of active substance Tebuconazole: EFSA Journal 2014; 12(1):3485; 88 pp.

For *Folsomia candida* as well as for *Hypoaspis aculeifer*, the long-term TER values calculated for BAS 750 F, 1,2,4-triazole and BAS 750 F as contained in BAS 750 01 F are above the Commission Regulation (EU) 546/2011 trigger of 5.

In conclusion, no unacceptable effects are expected for non-target soil meso- and macrofauna (other than earthworms) when BAS 750 01 F is applied according to the proposed use pattern.

B.9.8.3 Enantiomeric Ratio

The TGAI BAS 750 F is a 50:50 racemic enantiomer mixture consisting of the S-enantiomer Reg.No. 5934588 and the R-Enantiomer Reg.No. 5934591. The isomeric ratio of BAS 750 F was investigated in all relevant studies (i.e. where interactions with other chiral molecules could influence or select for degradation of one isomer over the other) representing relevant compartments and over various time points in the studies. No significant shifts or changes in isomeric ratio were observed throughout the duration of the studies investigated with the exception of two soils of the aerobic soil metabolism study. Here a slight shift of enantiomeric ratio from 50:50 to 45:55 was detected. All other investigated matrices, studies and measurements showed a 50:50 ratio throughout without any indication of a shift. The applicant has proposed the following consideration for the deviation in enantiomeric ratio:

"This slight change is not deemed a significant change and must be considered as background noise and within the methodological variation of such studies. Due to the above

mentioned investigations it can be concluded that the occurrence and degradation of the isomers are comparable in the environment. Therefore, exposure and subsequent risk assessments can be performed without considering the individual isomers.

Nevertheless, theoretical considerations can be made to address a potential shift of the enantiomers. In general, both enantiomers showed biological activity on all tested pathogens. Depending on the disease the (R)-enantiomer or the (S)-enantiomer was the more active compound (see Section CA 3.2 of the dossier). For soil organisms no toxicological data on the enantiomers alone are available. To evaluate a theoretical potential impact of such a shift, one may assume that the toxicological effects are due to one enantiomer only as an absolute worst case assumption. The most sensitive organism for the racemic 50:50 mixture in soil is the earthworm with a NOEC of 8 mg/kg dry soil observed in the chronic study. If one enantiomer would carry all activity the endpoint would be 4 mg/kg dry soil. Considering mixture toxicity, a shift of the active enantiomer from 50% to 55% would also cause a theoretical shift of 10% of the NOEC (i.e. from 8 to 7.3 mg/kg dry soil). This consideration suggests the potential shift being a minor uncertainty which has no impact on the overall conclusion of the soil risk assessment. In addition, the submitted field studies with the most sensitive soil organism (i.e. earthworm, see section CP 10.4.1.2) covers potential exposure under realistic conditions."

The RMS notes there is no toxicity data available for the individual toxicity of each enantiomer. However, under a worst case assumption that the shift in the enantiomeric ratio would reach 0:100 of the more toxic compound and under the worst case assumption that the more toxic enantiomer is responsible for all the toxicity, all the endpoints would be halved. Risk assessments based on the worst case assumptions are presented in Table B.9.8.3-1 below.

Table B.9.8.3-1: Summary of endpoints on earthworms and other soil macro-organism for BAS 750 F adjusted for worst case enantiomeric ratio and toxicity

Test substance	Test species	Endpoint	Value [mg/kg dry soil]	PEC_{soil} (ACCUMULATION) [mg/kg dry soil]	TER_{LT}	TER trigger
BAS 750 F	*Eisenia fetida*	$EC_{10\ corr}$	1.325	0.308	**4.3**	5
BAS 750 F in BAS 750 01 F		$NOEC_{corr}$	≥ 1.99	0.308	≥ 6.46	
BAS 750 F [#]	*Folsomia candida*	$NOEC_{corr}$	≥ 100	0.308	≥ 324.5	
BAS 750 F in BAS 750 01 F		$NOEC_{corr}$	> 6.0175	0.308	> 19.5	
BAS 750 F [#]	*Hypoaspis aculeifer*	$NOEC_{corr}$	≥ 250	0.308	≥ 812.5	
BAS 750 F in BAS 750 01 F		$NOEC_{corr}$	4.48	0.308	14.55	

[#] Study is presented in III CA B.9.4.2

There is an acceptable risk presented to all groups with the exception of earthworms based upon the worst case assumption, noting it is also acceptable for the formulation. Therefore some further consideration of the risk is required. Two earthworm field studies are available. Schulz L.,2015b (B.9.7.1/3) is considered sufficiently reliable to be used as a refinement (B.9.8.1), and indicates no toxic effects at an application rate of 6L BAS 750 01 F/ha. Hamberger A., 2015a B.9.7.1/2 is also considered sufficiently reliable to be used in risk assessments, and indicates no toxic effects at the maximum application rate of 8.0 L BAS 752 AM F/ha (800g BAS 750 F/ha and 500g BAS 700 F/ha).

It should be noted that the shift in enantiomeric ratio occurs over the 120 days of the soil test (KCA 7.1.1.1/001, Staudenmaier, H. and Dalkmann, P., (2015a)). The earthworm field studies are in excess of 120 days and hence exposure to the shift in ratio is considered likely to have occurred. As was stated above no adverse effects were observed at 6L BAS 750 01 F/ha and 8L BAS 752 AM F/ha, so it is unlikely that the shift in ratio would impact earthworms.

The risk to soil organisms from the enantiomeric ratio is considered acceptable from the shifts in the enantiomeric ratio.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Report: B.9.9/1
Persdorf M., 2014a
Effects of BAS 750 01 F on the activity of soil microflora (Nitrogen transformation test)
2014/1242736
Guidelines: OECD 216 (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm^3.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA): Sand content 55.8 % (USDA), pH 6.3, 1.50% C_{org} (of which microbial biomass comprised 2.37 %), 38.42% water holding capacity (WHC).

Test design: Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (C/N ratio = 13.2 / 1; concentration in soil 0.5%). Comparison of test substance treated soil with a non-treated soil. Sampling scheme: 0, 7, 14 and 28 days after treatment, sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to measurement. Soil samples of 10 g were taken and mixed with 50 mL 1M KCl solution before being centrifuged and the supernatant frozen prior to analysis. NH_4-nitrogen formed from organically bound nitrogen in the soil and NO_3-nitrogen from the nitrification process was determined by using an Autoanalyzer (Bran and Luebbe; LOQ (Nitrogen) = 0.32 mg/100 g dsw).

Endpoints: Effects on NO_3-nitrogen production 0, 7, 14 and 28 days after application.

Test concentrations: Control (deionised water), 4.77 and 23.83 mg BAS 750 01 F/kg dry soil (corresponding to an application rate of 3.6 and 18 L BAS 750 01 F/ha). The test substance was mixed with deionised water so that the test concentrations were prepared when the test solutions were mixed with 200 g soil (dry weight) using a hand stirrer.

Reference item: Dinoterb (purity: 98.0% ± 0.5% analysed). The reference item was tested in a separate study at rates of 6.80, 16.00 and 27.00 mg/kg dry soil. The reference item produced a stimulation of nitrogen transformation of +47.3%, +67.7%

and +35.1% at 6.80, 16.00 and 27.00 mg/kg dry soil, respectively, 28 days after application.

Test conditions: pH 6.0 - 6.1; measured water content: 16.27 –17.09 g/100 g dry soil (42.34 – 44.9 % WHC). Soil samples were incubated at 18.8 °C – 21.0 °C while stored in glass flasks in the dark. Test units were 500 mL glass flasks with screw caps that permitted air exchange.

Statistics: Descriptive statistics – mean nitrogen content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date. No analytical tests for statistically significant results were reported.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 750 01 F on nitrogen transformation could be observed at both test concentrations after 28 days. Only negligible deviations from the control of +0.4% (4.77 mg BAS 750 01 F/kg dry soil) and +2.0% (23.83 mg BAS 750 01 F/kg dry soil) were measured at the end of the 28-day incubation period (time interval 0-28). The results are summarised in Table B.9.9/1-1.

Table B.9.9/1-1: Effects BAS 750 01 F on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 of incubation

Time interval (days)	Control	4.77 mg BAS 750 01 F/kg dry soil, equivalent to 3.6 L/ha		23.83 mg BAS 750 01 F/kg dry soil, equivalent to 18 L/ha	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 -7 d)	+35.43	+34.27	-3.3	+35.40	-0.1
Loamy sand soil (0 - 14 d)	+47.57	+45.80	-3.7	+45.87	-3.6
Loamy sand soil (0 - 28 d)	+61.17	+61.43	+0.4	+62.40	+2.0

1) Based on NO_3-nitrogen production; - = inhibition; + = stimulation.

Validity Criteria
The study meets the validity criteria described in OECD 216:

- Coefficient of variation between replicate control samples was no more than 15 % (being 3.3 %)

III. CONCLUSION

Based on the results of this study, BAS 750 01 F caused no adverse effects (deviation from control < 25%, OECD 216) on the nitrogen transformation in a loamy sand soil tested up to a concentration of 23.83 mg BAS 750 01 F/kg dry soil, equivalent to a field application rate of 18 L BAS 750 01 F/ha.

RMS Comments
The study was carried out according to GLP and follows guideline OECD 216 with no significant deviations noted. The differences in nitrogen transformation rate between the control soil and the test

substance treated soils were checked by the RMS and did not exceed 25 % after 28 days. The recalculated results are summarised below:

	% EFFECT (negative (-) results indicate increase relative to control)			
Period (d)	0-7	7-14	14-21	21-28
4.77 mg/kg	3.29	4.95	-14.95	-1.11
23.83 mg/kg	0.09	13.74	-21.57	-3.41

The agreed endpoint considered suitable for use in the risk assessment is:
No adverse effects (< 25 %) on nitrogen transformation at a concentration of ≥ 23.83 mg f.p./kg dsw (18 L f.p./ha)

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

BAS 750 01 F is the representative formulation for the approval of the new fungicidal active substance BAS 750 F. BAS 750 01 F is an EC (emulsifiable concentrate) formulation, containing 100 g BAS 750 F/L intended for use in winter and spring cereals.

For the active substance BAS 750 F and the formulation BAS 750 01 F study endpoints are used for the risk assessment on soil microbial activity. Regarding the metabolite 1,2,4-triazole, the EU agreed endpoint is used for the risk assessment. All relevant data and assessments concerning the active substance BAS 750 F and the representative formulation BAS 750 01 F are provided below.

Table B.9.10-1: Soil microbial activity ecotoxicological endpoints for soil micro-organisms

Test substance	Test design [1)]	EU agreed endpoints	Endpoints used in risk assessment
BAS 750 F	C *	--	< 25% effects after 28 days at 2.53 mg/kg dry soil **
	N #	--	< 25% effects after 28 days at 2.53 mg/kg dry soil
1,2,4-triazole	N°	< 25% effects after 28 days up to and including 0.333 mg/kg dry soil	< 25% effects after 28 days up to and including 0.333 mg/kg dry soil
BAS 750 01 F	C **	--	< 25% effects after 28 days at 23.83 mg/kg dry soil (equivalent to 2.40 mg a.s./kg dry soil) **
	N	--	< 25% effects after 28 days at 23.83 mg/kg dry soil (equivalent to 2.40 mg a.s./kg dry soil)

1) C = Carbon transformation, N = Nitrogen transformation.

* Study summary is presented as additional information in Volume III CA Section B.9.7, as carbon transformation studies are no longer required according to EU Commission Regulation No.283/2013.

** Study summary is presented as additional information in section B.9.13 (this document), as carbon transformation studies are no longer required according to EU Commission Regulation No.283/2013.

[#] Study summary is presented in chapter Volume III CA Section B.9.5.
[°] the study has already been evaluated and EU agreed, e.g. Epoxiconazole, EFSA Scientific Report, 138, 2008, DAR, Vol. 3, B.9, 2006.

Toxicity

The toxicity of BAS 750 F, 1,2,4-triazole and BAS 750 F as contained in BAS 750 01 F to soil micro-organisms is summarised in the table below.

Table B.9.10-2: Toxicity of BAS 750 F and BAS 750 01 F to soil micro-organisms

Test substance	Endpoint	Endpoint (<25% effect) [mg/kg dry soil]	Reference
BAS 750 F [#]	Effects on nitrogen transformation	2.53	III CA B.9.5/1 Schulz L., 2015a
1,2,4-triazole		0.333	EFSA Scientific Report, 138, 2008
BAS 750 F in BAS 750 01 F		2.40	III CP B.9.9/1 Persdorf M., 2014a

[#] Study summary is presented in Volume III CA Section B.9.5.

Exposure

Table B.9.10-3: Proposed use pattern of BAS 750 01 F

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				BAS 750 F [g a.s./ha]	BAS 750 01 F [L/ha]
cereals	30 - 69	2	14	150	1.5

The exposure to soil organisms was estimated by calculating the maximum predicted environmental concentrations in soil (PEC_{soil}). For multiple applications, the worst-case maximum PEC_{soil} will be the one immediately after the final application. Regarding the PEC_{soil}, the worst-case use pattern of BAS 750 01 F envisages two applications to cereals with a maximum single dose rate of 1.5 L/ha, corresponding to 150 g BAS 750 F/ha. Since the worst-case DT_{90} values of BAS 750 F and 1,2,4-triazole exceed 365 days, $PEC_{soil, accu}$ values were calculated, assuming accumulation concentration and reflecting multi-year use. For details, see chapter III CP B.8.2.1.2. The resulting maximum PEC_{soil} values are presented in the table below

Table B.9.10-4: Worst-case PEC_{soil} values for BAS 750 F

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
BAS 750 F	0.080	0.308
1,2,4-triazole	< 0.001	< 0.001

Risk assessment

The risk assessment for soil microorganisms is summarised in the table below. The highest PECsoil value for each substance is compared with the endpoint. Where the PECsoil is lower than the concentration where < 25 % effects on nitrogen transformation rates are observed, an acceptable risk can be concluded.

Test substance	Endpoint	Endpoint (<25% effect) [mg/kg dry soil]	PECsoil (accumulation)	Acceptable risk? (Y/N)
BAS 750 F [#]	Effects on nitrogen transformation	2.53	0.308	Y
1,2,4-triazole		0.333	<0.001	Y
BAS 750 F in BAS 750 01 F		2.40	0.308	Y

The endpoints (<25 % effect concentration) exceed the PECsoil for both BAS 750 F and 1, 2,4-triazole. An acceptable risk can be concluded for the proposed uses of BAS 750 F.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.11.1 Summary of screening data

No screening studies on non-target plants have been performed, and none are required.

B.9.11.2 Testing on non-target plants

Report: B.9.11.2/1
Marquardt J., 2015a
BAS 750 01 F: A test to determine the effects on non-target plants
2014/1242738

Guidelines: OECD 208 (2006) - Seedling Emergence and Seedling Growth Test, EPA 850.4100 - Seedling Emergence and Seedling Growth (2012)

GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed).

B. STUDY DESIGN

Test species: Oilseed rape *(Brassica napus)*, lettuce (*Lactuca sativa)*, tomato (*Solanum lycopersicum.*), green cabbage (*Brassica oleracea* var. *sabellica)*, soybean

(*Glycine max*), carrot (*Daucus carota*), onion (*Allium cepa*), ryegrass (*Lolium multiflorum*), wheat (*Triticum aestivum*) and corn (*Zea mays*).

Test design: Dose-response design; 6 treatments (5 test substance rates, untreated control); 4 replicates per treatment, 2 pots/replicate, each pot with 5 seeds per pot for big plant species like oilseed rape, lettuce, tomato, soybean and corn or 1 pot/replicate with 10 seeds each for small species like green cabbage, carrot, onion, ryegrass and wheat (40 plants, per species, per treatment group); greenhouse cultivation; BAS 750 01 F was applied to the soil pre-emergence using a laboratory spray cabin at a water volume of 200 L/ha; assessments for seedling emergence, growth stage and phytotoxicity were done 7, 14 and 21 (± 1) days after application (DAA) (tomato: 9, 16 and 22 DAA; onion: 12, 18 and 25 DAA due to slower seedling emergence); plant dry weight and plant height of all species tested was determined at study termination.

Test Soil: PS3-2014 soil medium - Loamy Sand (80.6 % sand), 1.45 % C_{org}, pH 6.85

Endpoints: NOER, ER_{25}, ER_{50}.

Test rates: Control (deionised water), 93.75, 187.5, 375.0, 750.0 and 1500.0 mL BAS 750 01 F/ha. Aliquots of a stock solution of the test substance were further diluted with deionised water up to 500 mL to obtain the desired concentration range. The concentrations of the control and highest test rate solution were analysed by the HPLC method and recovery levels were found to be between 102.1 – 104.9 % in the latter, supporting the use of nominal values for the concentrations of the application solutions. The laboratory sprayer used to apply the solutions was calibrated prior to application by weighing the deposit of water left by the sprayer on plates of known dry weight. The amount of water deposited by the sprayer was weighed afterwards and found to be within 10 % of the desired rate (200 L/ha; deviation ranged between -4.57 and -0.46 %).

Test conditions: Daily mean temperature (onion): 22.5 °C (19.1 °C – 27.0 °C); daily mean humidity (onion): 48.0% (40.0% – 69.0%); daily mean temperature (all other species): 21.5 °C (20.3 °C – 23.9 °C); daily mean humidity (all other species): 56.0% (40.0% – 65.0%); photoperiod: 16 h light: 8 h dark; additional light when outdoor illumination was less than 10 klx. Test units were 15 cm diameter plastic pots filled with 120 g clay pebbles and 990 g fresh soil medium.

Statistics: Descriptive statistics; Data tested for normal distribution with Kolmogoroff-Smirnoff Test ($\alpha = 0.05$). ANOVA followed by Dunnett`s t-test (for variance homogeneity) or Bonferroni-Welch t-test (for variance heterogeneity) ($\alpha = 0.05$). Probit analysis for calculation of ER_{25} and ER_{50}. The calculations were carried out using Microsoft® Excel (2010 SP2) and ToxRat Standard 2.10.05.

II. RESULTS AND DISCUSSION

The pre-emergence application of BAS 750 01 F resulted in no clear treatment-related symptoms of phytotoxicity (average phytotoxicity score was ≤ 5.0 %, apart from onion at 8 %) for all tested species. Also, no unacceptable adverse effects on seedling emergence and plant height were observed for all 10 tested species at test termination, each with a NOER of ≥ 1500.0 mL BAS 750 01 F/ha. Statistically significant effects on plant dry weight were only observed in wheat at 1500.0 mL BAS 750 01 F/ha

(Dunnett's t-test, $\alpha = 0.05$), resulting in a NOER of 750.0 mL BAS 750 01 F/ha.

The results are summarised in Table B.9.11.2/1-1 and Table B.9.11.2/1-2.

Table B.9.11.2/1-1: Effects of BAS 750 01 F on phytotoxicity, plant height, plant dry weight and seedling emergence 21 DAA (22 DAA for tomato and 25 DAA for onion)

Treatment [mL/ha]	Oilseed rape	Lettuce	Tomato	Green cabbage	Soybean	Carrot	Onion	Rye grass	Wheat	Corn
Phytotoxic damages [%]										
Control	0	0	0	0	0	0	0	0	0	0
93.75	0	0	0	0	0	0	0	0	0	0
187.5	0	0	0	0	0	0	0	0	0	0
375.0	0	4	0	0	0	0	3	0	0	0
750.0	4	5	0	0	0	0	0	0	0	0
1500.0	1	3	0	0	0	0	8	0	0	0
Seedling emergence rate [% deviation to control]										
93.75	-5	3	-5	0	-3	-10	10	-13	-3	0
187.5	0	3	-10	0	0	-6	-3	0	3	0
375.0	0	-5	0	3	-5	3	3	-5	5	0
750.0	-3	-8	-10	0	0	-13	28	-3	-5	-3
1500.0	-8	-10	-3	-3	-3	0	-10	0	3	0
Plant height [% deviation to control]										
93.75	9	11	1	5	3	4	-4	-5	-2	0
187.5	-1	11	3	-2	-5	11	1	-4	-3	0
375.0	1	-2	-6	-2	1	10	-12	-8	-9	0
750.0	2	-2	4	-2	2	0	-6	1	0	1
1500.0	0	-6	9	3	1	5	-19	-2	-7	2
Plant dry weight [% deviation to control]										
93.75	-7	22	-1	4	0	-12	-3	-17	-10	3
187.5	10	43	6	-7	-9	-8	22	-11	-5	3
375.0	-17	17	-15	-3	-3	-28	-9	-13	-12	6
750.0	-11	20	-5	-17	5	-10	2	-1	-11	8
1500.0	0	-18	16	1	-5	-18	-12	-5	-16 *	6

* Statistically significantly different compared to the control (Dunnett's t-test, $\alpha = 0.05$).

Table B.9.11.2/1-2: NOER, ER_{25} and ER_{50} of BAS 750 01 F for non-target plants 21 DAA (22 DAA for tomato and 25 DAA for onion)

Treatment [mL/ha]	Oilseed rape	Lettuce	Tomato	Green cabbage	Soybean	Carrot	Onion	Rye grass	Wheat	Corn
Phytotoxicity										
NOER	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0
Seedling emergence										
NOER	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0
ER_{25}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
ER_{50}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
Plant height										
NOER	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0
ER_{25}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
ER_{50}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
Plant dry weight										
NOER	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	750.0	≥ 1500.0
ER_{25}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
ER_{50}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0

Validity Criteria

The study met the validity criteria specified in OECD 208:

- Control seedling emergence was not less than 70 % in all replicates (being minimum 73 % for onion)
- Control Seedlings did not exhibit any phytotoxic effects
- Environmental conditions for each species were identical between replicates
- Control survival of emerged seedlings was not less than 90 % (being minimum 90 % (carrot))

III. CONCLUSION

Pre-emergence application of BAS 750 01 F under worst-case greenhouse conditions did not result in treatment-related symptoms of phytotoxicity for any of the tested plant species. No adverse effects on seedling emergence and plant height were observed for all plant species tested up to and including 1500.0 mL BAS 750 01 F/ha. Statistically significant effects on dry weight were observed for wheat at an application rate of 1500.0 mL BAS 750 01 F/ha.

The ER_{50} value based on seedling emergence, plant height and dry weight was > 1500.0 mL BAS 750 01 F/ha for all tested plant species (the highest rate tested). The NOER for phytotoxicity, seedling emergence and plant height was ≥ 1500.0 mL BAS 750 01 F/ha, and the NOER for plant dry weight was 750.0 mL BAS 750 01 F/ha.

RMS Comments

The study was carried out according to GLP and follows the guideline OECD 208 with no significant deviations. The study report notes that there were a number of deviations from guidance with regards to relative humidity, with levels less than the recommended minimum of 45 % (mean minimum 40 % for seven days after application for Onion, mean minimum 40.34 % for two days for all other species). This is not considered to have had a significant effect on the results as the study validity criteria were met.

The apparent large deviations from the control that do not result in statistically significant results indicate that the study design lacks sensitivity. However as no effects greater than 50 % were

observed, and as there appears to be no dose-response relationship for any of the tested species, the ER_{50} value derived from this study is considered appropriate for risk assessment. That said there is uncertainty regarding the NOER values derived.

The agreed endpoint considered suitable for use in the risk assessment is:
$ER_{50} \geq$ 1500 mL BAS 750 01 F/ha

Report: B.9.11.2/2
Marquardt J., 2015 b
BAS 750 01 F: A test to determine the effects on non-target plants
2014/1242739 (AgroScience study number AS413) and Final Report Amendment no. 1 (DOC ID 2016/1280262 ; 13/10/2016)

Guidelines: OECD 227 Terrestrial Plant Test: Vegetative Vigour Test (July 2006), EPA 850.4150 - Vegetative Vigour (2012)

GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100.0 g/L nominal (98.9 g/L analysed); density: 0.993 g/cm³.

B. STUDY DESIGN

Test species: Oilseed rape *(Brassica napus)*, lettuce (*Lactuca sativa)*, tomato (*Solanum lycopersicum*), green cabbage (*Brassica oleracea)*, soy bean (*Glycine max*), carrot (*Daucus carota*), onion (*Allium cepa*), ryegrass (*Lolium multiflorum*), wheat (*Triticum aestivum*) and corn (*Zea mays*).

Test design: Dose-response design; 6 treatments (5 test substance rates, 1 control); 5 replicates per treatment; 2 pots/replicate, each pot with 3 plants per pot for big species like oilseed rape, lettuce , tomato, soybean and corn or 1 pot/replicate with 6 plants per pot for small species like green cabbage, carrot, onion, ryegrass and wheat (30 plants per treatment group, per species); greenhouse cultivation; post-emergence application at a 2 to 4 leaf growth stage using a laboratory spray cabin at a water volume of 200 L/ha; assessment for phytotoxicity was done 7, 14 and 21 days after application (DAA); plant dry weight and plant height was determined at study termination 21 DAA.

Test soil: PS3-2014 soil medium – slightly loamy sand soil (80.6 % sand), C_{org} = 1.45 %, pH = 6.85.

Endpoints: NOER, ER_{25}, ER_{50}.

Test rates: Control (deionised water), 93.75, 187.5, 375.0, 750.0 and 1500.0 mL BAS 750 01 F/ha. Aliquots of a stock solution of the test substance were further diluted with deionised water up to 500 mL to obtain the desired concentration range. The concentrations of the untreated control and the highest test rate were analysed by the HPLC method and recovery levels were found to be 102.3 % in the latter, supporting the use of nominal values for the concentrations of the application solutions. The laboratory sprayer used to

apply the solutions was calibrated prior to application by weighing the deposit of water left by the sprayer on plates of known dry weight. The amount of water deposited by the sprayer was weighed afterwards and found to be within 10 % of the desired rate (200 L/ha; deviation ranged between -8.8 and 8.33 %).

Test conditions: Daily mean temperature: 19.1 °C – 23.0 °C; daily mean humidity: 45.0% – 77.0%; photoperiod: 16 h light: 8 h dark; additional light when outdoor illumination was less than 10 klux. Test units were 15 cm-diameter plastic pots filled with 120 g clay pebbles and 990 g soil medium (fresh weight).

Statistics: Descriptive statistics; Data tested for normal distribution with Kolmogoroff-Smirnoff Test ($\alpha = 0.05$). ANOVA followed by Dunnett`s t-test (for variance homogeneity) or Bonferroni-Welch t-test (for variance heterogeneity) ($\alpha = 0.05$). The calculations were carried out using Microsoft® Excel (2010 SP2) and ToxRat Standard 2.10.05.

II. RESULTS AND DISCUSSION

After exposure to BAS 750 01 F, no treatment-related symptoms of phytotoxicity (average phytotoxicity score < 5 % in every replicate) were observed for any of the tested plant species. Further, no statistically significant adverse effects on plant height or plant dry weight were assessed, resulting in a NOER of ≥ 1500.0 mL BAS 750 01 F/ha.

The results are summarised in Table B.9.11.2/2-1 and Table B.9.11.2/2-2.

Table B.9.11.2/2-1: Effect of BAS 750 01 F on phytotoxicity, plant height and plant dry weight 21 DAA

Treatment [mL/ha]	Oilseed rape	Lettuce	Tomato	Green cabbage	Soy bean	Carrot	Onion	Rye grass	Wheat	Corn
Phytotoxic damages [%]										
Control	0	0	0	0	0	0	0	0	0	0
93.75	0	0	0	0	0	0	0	0	0	0
187.5	0	0	0	0	0	0	0	0	0	0
375.0	0	0	0	0	0	0	0	0	0	0
750.0	0	0	0	0	0	0	0	0	0	0
1500.0	0	0	0	0	3	0	0	0	0	0
Plant height [% deviation to control]										
93.75	5	1	-1	4	0	-2	4	3	0	1
187.5	4	-2	0	3	12	-3	2	1	-1	-1
375.0	-1	-5	2	-3	6	-1	4	-8	-4	0
750.0	-4	-5	5	-3	6	1	-3	1	1	-1
1500.0	2	-2	1	0	2	1	-1	0	3	1
Plant dry weight [% deviation to control]										
93.75	0	-2	3	12	2	4	3	-14	-6	-7
187.5	-4	-1	6	22	7	8	-2	-13	0	-8
375.0	0	-4	-5	11	16	3	7	-21	-1	-6
750.0	-8	2	-15	-10	8	9	-6	-16	1	-6
1500.0	-3	-1	-14	3	2	10	-3	-13	5	-3

Table B.9.11.2/2-2: NOER, ER_{25}, and ER_{50} of BAS 750 01 F for non-target plants 21 DAA

Treatment [mL/ha]	Oilseed rape	Lettuce	Tomato	Cabbage	Soy bean	Carrot	Onion	Rye grass	Wheat	Corn
Phytotoxicity										
NOER	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0
Plant height										
NOER	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0
ER_{25}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
ER_{50}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
Plant dry weight										
NOER	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0	≥ 1500.0
ER_{25}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0
ER_{50}	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0	> 1500.0

Validity Criteria
The study meets the following validity criteria outlined in OECD 227:

- Control mortality and exhibition of phytotoxic symptoms were both nil
- Environmental conditions for all species were identical
- Seedling emergence was no less than 70 %, ranging from 75-100 % across all species (Final Report Amendment no. 1)

III. CONCLUSION

Post-emergence application of BAS 750 01 F under worst-case greenhouse conditions resulted in no treatment-related symptoms of phytotoxicity for all tested plant species. Thus, the NOER was ≥ 1500.0 mL BAS 750 01 F/ha for all tested plant species.
The ER_{50} value based on plant dry weight and height was > 1500.0 mL BAS 750 01 F/ha for all tested plant species (the highest rate tested).

RMS Comments
The study was carried out according to GLP and follows the guideline OECD 227 with no significant deviations. The apparent large deviations from the control that do not result in statistically significant results indicate that the study design lacks sensitivity. However as no effects greater than 25 % were observed, this is not thought to have had an adverse impact on the study. As there appears to be no dose-response relationship for any of the tested species, the ER_{50} value derived from this study is considered appropriate for risk assessment.

The agreed endpoint considered suitable for use in the risk assessment is:
$ER_{50} \geq 1500$ mL BAS 750 F/ha

B.9.11.3 Extended laboratory studies on non-target plants

An acceptable risk to non-target plants was concluded using first tier laboratory study data. Therefore no extended laboratory studies are required.

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

An acceptable risk to non-target plants was concluded using first tier laboratory study data. Therefore no semi-field or field studies are required.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

Toxicity

A summary of the potential effects of BAS 750 01 F on seedling emergence and vegetative vigor in greenhouse environments is provided in the table below.

Table B.9.12-1: Summary of effects on terrestrial non-target plants following exposure to BAS 750 01 F

Test substance	Test system	Test species	EU agreed endpoints	Endpoints used in risk assessment	Reference
BAS 750 01 F	greenhouse, seedling emergence dose-response test	oilseed rape, lettuce, tomato, cabbage, soybean, carrot, onion, ryegrass, wheat, corn	--	ER_{50} > 1.5 L/ha (all species)	2014/1242738
BAS 750 01 F	greenhouse, vegetative vigor dose-response test	oilseed rape, lettuce, tomato, cabbage, soybean, carrot, onion, ryegrass, wheat, corn	--	ER_{50} > 1.5 L/ha (all species)	2014/1242739

Exposure

Table B.9.12-2: Proposed use pattern of BAS 750 01 F

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				BAS 750 F [g a.s./ha]	BAS 750 01 F [L/ha]
cereals	30 - 69	2	14	150	1.5

Effects on non-target plants are of concern in the off-field environment where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA [*Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880*

(25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain] from the spray-drift predictions of Ganzelmeier & Rautmann [Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain]. The maximum application was considered as required by the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002).

This is due to factors such as plant growth, which may reduce residues per unit area between multiple applications. For a single application to cereals, 2.77% of the application rate is assumed to reach areas at 1 m from the edge of the crop.
The highest single application rate is 1.5 L BAS 750 01 F/ha, giving a maximum off-field predicted environmental rate ($PER_{off-field}$) of 0.042 L BAS 750 01 F/ha.

Risk assessment

BAS 750 01 F is a fungicide and is therefore not expected to have any significant herbicidal activity. However, a dose-response study examining the effects on pre-emergence of non-target plants resulted in an ER_{50} value of > 1.5 L BAS 750 01 F/ha for all 10 tested species (see DocID 2014/1242738).
A dose response test, studying the effects on post-emergence of non-target plants, was conducted and resulted in an ER_{50} value of > 1.5 L BAS 750 01 F/ha for all 10 tested species (see DocID 2014/1242739).
According to the Terrestrial Guidance Document [*Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under council directive 91/414/EEC. SANCO/10329/2002. 17 October 2002*] the risk to non-target plants should be considered acceptable if less than 50% effect on all species tested is seen at the maximum single application rate. In the seedling emergence and vegetative vigor tests with BAS 750 01 F (please see B.9.6.2 for detailed summaries), the ER_{50} exceeded the maximum intended application rate of BAS 750 01 F. It can therefore be concluded that the proposed use of BAS 750 01 F poses no unacceptable risk to non-target plants.

Moreover, the calculated maximum $PER_{off-field}$ of 0.042 L BAS 750 01 F/ha is considerably below the level found to have no effects on the non-target plants. Based on the drift values, it can be concluded that the proposed use of BAS 750 01 F poses no unacceptable risk to non-target plants. This is confirmed by the calculation of TER values in the table below:

Test substance	Test system	Test species	Endpoints used in risk assessment	Drift rate (L BAS 750 01 F/ha)	TER
BAS 750 01 F	greenhouse, seedling emergence dose-response test	oilseed rape, lettuce, tomato, cabbage, soybean, carrot, onion, ryegrass, wheat, corn	ER_{50} > 1.5 L/ha (all species)	0.042	35.7
BAS 750 01 F	greenhouse, vegetative vigor dose-response test	oilseed rape, lettuce, tomato, cabbage, soybean, carrot, onion, ryegrass, wheat, corn	ER_{50} > 1.5 L/ha (all species)		

The TER for both seedling emergence and vegetative vigour exceed the trigger value of 5 with a high margin of safety. In conclusion, an acceptable risk to terrestrial non-target plants in off-field areas can be concluded for the proposed uses of BAS 750 F.

B.9.13 EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

This section contains study data submitted in support of the application that is no longer a requirement for the risk assessment of the active substance according to Commission regulation EU no. 283/2013, and are summarised here to provide additional information.

Report: B.9.13/1
Schulz L., 2015a
Effects of BAS 750 01 F on the activity of soil microflora (carbon transformation test)
2015/1000993
Guidelines: OECD 217 (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F; batch no. FD-140113-0006; content of a.s.: BAS 750 F: 100 g/L nominal (98.9 g/L analysed), density: 0.993 g/cm^3.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA): Sand content 52.9 %, pH 6.1, 1.32% C_{org} (of which microbial biomass comprised 3.10 %), 39.54 g/100 g dry soil water holding capacity (WHC).

Test design: Determination of carbon transformation in soil after addition of glucose (concentration in soil 0.6%). Comparison of test substance treated soil with control (non-treated soil). Three replicates per treatment and concentration. Per replicate, 100 g samples (soil dry weight) were taken at 0 (3 hours after application), 7, 14 and 28 days after treatment. The samples were mixed with glucose and filled into 500 mL reaction flasks. Afterwards, small vessels containing 1M NaOH solution were also placed into the reaction flasks, which were then closed and connected to a "BSB-digi" respirometer system. This was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: Sub-samples were withdrawn from the bulk batches and subjected to measurement.

Endpoints: Effects on O_2 consumption 0, 7, 14 and 28 days after application.

Test concentrations: Control, 4.77 and 23.83 mg BAS 750 01 F/kg dry soil (corresponding to an application rate of 3.6 and 18.0 L BAS 750 01 F/ha). 1 kg soil (dry weight) per vessel was weighed and mixed with a solution of the test substance diluted in appropriate quantities of deionised water, using a laboratory mixer.

Reference item: Dinoterb (purity: 98.0% ± 0.5% analysed). The reference item was applied at a rate of 6.80, 16.00 and 27.00 mg/kg dry soil in a separate study (6th January – 3rd February 2015). The reference item caused an inhibition of carbon transformation of -30.1% and -39.6% at 16.00 and 27.00 mg/kg dry soil, respectively, 28 days after application.

Test conditions: Measured water content: 16.83 – 17.89 g/100 g dry soil (42.58 – 45.23 % WHC – water content was checked after application of the test substance and adjusted each week to the required range of 40 – 50 % WHC); pH 5.9 – 6.0. Soil samples were incubated at 19.2 °C – 21.5 °C while stored in steel vessels in the dark. Test units were 4 L steel vessels, with lids that permitted air exchange

Analysis: Cumulative oxygen consumption, calculation of standard deviation and coefficient of variation, calculation of the deviation from the control for each sampling date.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 750 01 F on carbon transformation in soil could be observed at both test substance concentrations after 28 days. Only negligible deviations from the control of -2.9% (test concentration of 4.77 mg BAS 750 01 F/kg dry soil) and +1.4% (test concentration of 23.83 mg BAS 750 01 F/kg dry soil) were measured at the end of the 28 day incubation period. The results are summarised in Table B.9.13/1-1.

Table B.9.13/1-1: Effects of BAS 750 01 F on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control	4.77 mg BAS 750 01 F/kg dry soil, equivalent to 3.6 L/ha		23.83 mg BAS 750 01 F/kg dry soil, equivalent to 18.0 L/ha	
	O_2 consumption [mg/kg dry soil/h]	O_2 consumption [mg/kg dry soil/h]	Deviation from control [%][1)]	O_2 consumption [mg/kg dry soil/h]	Deviation from control [%][1)]
Loamy sand soil (0 days)	14.70	15.02	+2.1	15.97	+8.6
Loamy sand soil (7 days)	16.34	16.36	+0.2	16.45	+0.7
Loamy sand soil (14 days)	14.30	14.42	+0.9	14.08	-1.5
Loamy sand soil (28 days)	13.76	13.36	-2.9	13.96	+1.4

1) Based on O_2 consumption; - = inhibition, + = stimulation

Validity Criteria

The study fulfilled the validity criteria specified in OECD 217:

- The coefficients of variation in the control were no more than 15 % (being max. 2.7 %)

III. CONCLUSION

Based on the results of this study, BAS 750 01 F caused no adverse effects (deviation from control < 25.0 %, OECD 217) on carbon transformation in a loamy sand soil tested up to a concentration of 23.83 mg BAS 750 01 F/kg dry soil, equivalent to a field application rate of

18.0 L BAS 750 01 F/ha.

RMS Comments
This study was conducted to GLP and follows OECD 217 with no deviations noted. It is no longer a requirement under EC 1107/2009, so has been omitted from the risk assessment, but may provide useful additional information.

The agreed endpoint considered suitable for use in the risk assessment is:
< 25 % effects on carbon transformation at 23.83 mg f.p./kg dsw (18 L f.p./ha), the highest concentration tested.

Report:	B.9.13/2 Straube D., 2015a BAS 750 01 F: Acute toxicity to the earthworm *Eisenia fetida* in artificial soil 2015/1188048
Guidelines:	OECD 207 (1984), ISO 11268-1 (2012)
GLP:	yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 01 F, batch FD-140113-0006, content of a.s.: BAS 750 F: 100 g/L nominal (98.9 g/L analysed).

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida)*, adult worms (with clitellum and weight 300 - 600 mg), age: approximately 8-9 months old; source: iBacon GmbH, in-house culture. Acclimatised for 1 day prior to study start under test conditions.

Test design: 14 days exposure in treated artificial soil (10% peat); different concentrations of the test substance were mixed homogeneously into the soil filled in glass vessels before the earthworms were introduced on top of the soil; 6 treatment groups (5 test substance concentrations, control); 4 replicates/treatment with 10 worms each. Assessment of worm mortality was conducted on days 7 and 14, measurement of weight change as sub-lethal parameter after 14 days.

Endpoints: LC_{50} (50% mortality of earthworms after exposure over 14 days), behavioural effects, weight change.

Reference item: Chloroacetamide. The effects of the reference item were investigated in a separate study (August 2014). The LC_{50} was determined to be 25.3 mg a.s./kg dsw. This lies within the range specified in ISO-11268-1 (1993) – 20-80 mg a.s./kg, thereby confirming the sensitivity of the test system.

Test concentrations: Control (deionised water), 62.5, 125, 250, 500 and 1000 mg BAS 750 01 F/kg dry soil (nominal). A stock solution of 4 mg test substance/g was prepared and appropriate quantities were added to 2050 g

(dry weight) artificial soil to prepare the range of test concentrations. Actual test concentrations did not deviate more than 5 % from nominal.

Test conditions: Artificial soil according to OECD 207; 10% sphagnum peat, 20 % Kaolin clay, 69.5 % quartz sand, 0.5 % $CaCO_3$, mixed with deionised water and, post-treatment, allocated to 1 L glass jars in 500 g portions (dry weight) ; pH 5.8 at test initiation, pH 5.9 - 6.4 at test termination; water content 50.8% - 52.6% of maximum water holding capacity (WHC) at test initiation, 48.2% - 51.7% of maximum WHC at test termination; temperature: 20 °C ± 2 °C; light intensity 400 lux - 800 lux; continuous illumination.

Statistics: Descriptive statistics, Fisher's exact test with Bonferroni correction for mortality (one-sided greater, $\alpha = 0.05$). Data on body weight changes were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test and Levene's test ($\alpha = 0.05$), respectively, and comparison between the treated groups and the control was carried out using Williams t-test (two sided, $\alpha = 0.05$). Software used was ToxRat Professional, Version 2.10.05, ToxRat® Solutions GmbH.

II. RESULTS AND DISCUSSION

After 14 days of exposure, no mortality was observed in the control and up to and including the concentration of 500 mg/kg dry soil. At 1000 mg/kg dry soil mortality was 100% (statistically significant, Fisher's exact test, $\alpha = 0.05$, one sided greater).
Reduction in worm biomass was measured in each treated group as well as in the control. Statistically significant differences in weight change were observed at test substance concentration of 500 mg/kg dry soil (Williams t-test, two-sided, $\alpha = 0.05$). At the highest test concentration of 1000 mg/kg dry soil weight changes could not be determined, as mortality was 100%. No behavioural abnormalities could be observed. The results are summarised in Table B.9.13/2-1.

Table B.9.13/2-1: Effect of BAS 750 01 F on earthworm (*Eisenia fetida)* mortality and biomass (14 d)

BAS 750 01 F [mg/kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality [%]	0.0	0.0	0.0	0.0	0.0	100 *
Weight change [%]	-9.4	-9.2	-9.7	-13.7	-20.2 *	n.d.
Endpoints [mg BAS 750 01 F/kg dry soil]						
$NOEC_{weight}$	250					
$NOEC_{mortality}$	500					
LC_{50}	> 500					

* = Statistically significant differences when compared to the control (Fisher's exact test, Williams t-test, $\alpha = 0.05$).

Validity Criteria
The study fulfils the validity criteria described in OECD 207

- Control mortality did not exceed 10 % at the end of the test (being 0 %).

II. CONCLUSION

In a 14 day toxicity study on earthworms (*Eisenia fetida)* with BAS 750 01 F the LC_{50} was determined to be > 500 mg/kg dry soil. The overall NOEC related to biomass was 250 mg BAS 750 01 F/kg dry soil.

RMS Comments
The study was carried out according to GLP and follows guideline OECD 207 with no deviations noted. Acute earthworm studies are not a requirement for risk assessment of the soil compartment under regulation EC 1107/2009, however they may provide useful additional information.

The agreed endpoint considered suitable for use in the risk assessment is:
14 day LC_{50} ≥500 mg f.p./kg dsw (equivalent to 49.5 mg a.s./kg dsw, based on the density of the formulation being 0.993 g/cm^3 and the concentration of the active substance being 98.9 g/L)

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Although studies are provided for carbon transformation and acute toxicity to earthworms, these studies are no longer required for the risk assessment for soil micro-organisms and soil meso- and macro-fauna, respectively. These studies therefore provide useful additional information. No further risk assessment is required.

B.9.14.1 Risk Assessment for biological methods for sewage treatment

Table B.9.14.1-1: Table of endpoints used to assess risk to biological sewage treatment

Organism	Test substance	Test type	Endpoint	Reference (Author, Date)
Activated sewage sludge	BAS 750 F	OECD 209 (2010)	3 h EC_{50} ≥ 1000 mg a.s./L	Hammer S., 2014a

Risk assessment

The risk to biological methods for sewage treatment is considered acceptable. The EC_{50} produced in the activated sewage sludge test (Hammer S., 2014a) was greater than 1000 mg a.s./L. This suggests limited risk to sewage treatment facilities. Additionally the EC_{50} is > 3000 times greater than the FOCUS step 1 initial PEC_{SW} (50.25 µg/L). Dilution prior to reaching sewage treatment works may also be expected to reduce the risk further.

B.9.15. REFERENCES 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	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.9.3.1/1	[redacted]	2014a	BAS 750 01 F - Rainbow trout, acute toxicity test 2014/1117112 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.3.1/2	[redacted]	2015a	BAS 750 BS F (blank formulation of BAS 750 01 F) - Rainbow trout, acute toxicity test 2015/1001875 [redacted] yes Unpublished	Yes	No	Data for first Approval	BASF
B.9.3.1/3	Turek T.	2015a	BAS 750 01 F - Daphnia magna acute immobilization test 2014/1117111 Institute of Industrial	No	Yes	Data for first Approval	BASF

			Organic Chemistry, Pszczyna, Poland yes Unpublished				
B.9.3.1/4	Brzozowska -Wojoczek K.	2015a	BAS 750 BS F (blank formulation of BAS 750 01 F) - Daphnia magna, acute immobilization test 2015/1177056 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	No	Data for first Approval	BASF

B.9.3.1/3	Turek T.	2015b	BAS 750 01 F - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2014/1117110 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.5.1/1	Franke M.	2015a	Acute toxicity of BAS 750 01 F to the honeybee Apis mellifera L. under laboratory conditions 2014/1242741 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.5.1/2	Franke M.	2015a	Acute toxicity of BAS 750 01 F to the honeybee Apis mellifera L. under laboratory conditions 2014/1242741 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.	No	Yes	Data for first Approval	BASF

			yes Unpublished				
B.9.5.2/1	Fallowfield L.	2015a	A rate-response laboratory test to determine the effects of BAS 750 01 F on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) 2014/1242742 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.5.2/2	Stevens J.	2015a	A rate-response laboratory test to determine the effects of BAS 750 01 F on the parasitic wasp Aphidius rhopalosiphi (Hymenoptera, Braconidae) 2014/1242743 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.5.2/3	Fallowfield L.	2015b	A rate-response extended laboratory test to determine the effects of BAS 750 01 F on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae)	No	Yes	Data for first Approval	BASF

			2015/1020207 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished				
B.9.5.2/4	Stevens J.	2015b	A rate-response extended laboratory test to determine the effects of BAS 750 01 F on the parasitic wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) 2014/1242744 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.5.2/5	Vaughan R.	2015a	A rate-response extended laboratory test to evaluate the effects of fresh residues of BAS 750 01 F on the green lacewing Chrysoperla carnea (Neuroptera, Chrysopidae) 2015/1020206 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	Yes	Data for first Approval	BASF

B.9.7.1/1	Ganβmann M.	2015a	Effects of BAS 750 01 F on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2015/1000884 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.7.1/2	Hamberger A.	2015a	BAS 752 AM F - A field study to investigate effects on earthworm fauna in Southern Germany 2015/1000261 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.7.1/3	Schulz L.	2015a	Effects of BAS 750 01 F on earthworms under field conditions 2015/1000163 BioChem agrar Labor fuer biologische und chemische Analytik GmbH,	No	Yes	Data for first Approval	BASF

			Gerichshain, Germany Fed.Rep. yes Unpublished				
B.9.7.2/1	Friedrich S.	2015a	Effects of BAS 750 01 F on the reproduction of the collembolan Folsomia candida 2015/1000885 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.7.2/2	Ganβmann M.	2015b	Effects of BAS 750 01 F on reproduction of the predatory mite Hyposaspis aculeifer in artificial soil with 5% peat 2014/1242737 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.7.2/2a	Ganβmann M.	2015c	1 st Final report amendment: Effects of BAS 750 01 F on reproduction of the predatory mite Hyposaspis aculeifer in	No	Yes	Data for first Approval	BASF

			artificial soil with 5% peat 2015/1035024 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished				
B.9.9/1	Persdorf M.	2014a	Effects of BAS 750 01 F on the activity of soil microflora (Nitrogen transformation test) 2014/1242736 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.11.2/1	Marquardt J.	2015a	BAS 750 01 F: A test to determine the effects on non-target plants 2014/1242738 RLP AgroScience GmbH, Neustadt/Weinstrasse, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.11.2/2	Marquardt J.	2015b	BAS 750 01 F: A test to	No	Yes	Data for first	BASF

			determine the effects on non-target plants 2014/1242739 Rheinland Pfalz AgroScience GmbH, Neustadt/Weinstrasse, Germany Fed.Rep. yes Unpublished			Approval	
B.9.13/1	Schulz L.	2015b	Effects of BAS 750 01 F on the activity of soil microflora (carbon transformation test) 2015/1000993 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.13/2	Straube D.	2015a	BAS 750 01 F: Acute toxicity to the earthworm Eisenia fetida in artificial soil 2015/1188048 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF